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Determination of the *In Vitro* and *In Vivo* Activity of Compounds Tested Against Punta Toro Virus.

Annual Report

Robert W. Sidwell, Ph.D.

December 20, 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD 17-86-C-6028

Utah State University
Logan, Utah 84322

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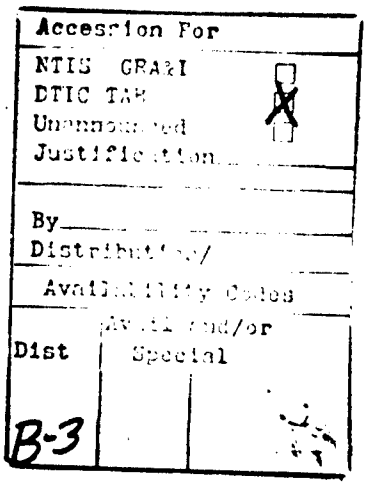

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This combined antiviral effect appeared to be synergistic. **Effect of the Combination of AVS01 and AVS1754 on *In Vivo* Punta Toro Virus Infections:** Treatment of Adames PTV-infected mice with a combination of AVS01 administered p.o. twice daily for 5 days beginning 24 hr after virus inoculation and the immunomodulator AVS1754 administered i.p. in a single injection 24 hr after virus inoculation was considered slightly to moderately more effective than using either alone. **Effect of a Combination of AVS206 and AVS1767 on In Vivo Punta Toro Virus Infections:** Treatment with AVS206 administered p.o. twice daily for 5 days beginning 18 hr after virus inoculation combined with the immunomodulator AVS1767 injected s.c. a single time 48 hr after virus inoculation had a definite enhanced effect on *in vivo* Adames PTV infections. This was seen as reductions in liver score, SGOT, SGPT, serum virus titers and liver virus titers. **Effects of Treatment with AVS206 on Delayed Infection Parameters in Punta Toro Virus-Infected Mice:** AVS206 administered p.o. twice daily for 3 days was highly effective vs Adames PTV infections in mice, and, once treatment was terminated, detectable infectious virus did not return to either livers or sera from surviving mice up to 4 days later. **Infectivity of Balliet Strain PTV Inoculated Intracerebrally into Balb/c and Swiss Webster Mice:** Both Balb/c and Swiss Webster mice were approximately equally susceptible to i.c. challenge with the Balliet strain of PTV, and was considered acceptable for anti-PTV studies. **Effect of Intravenous Therapy on Intracerebrally Induced Punta Toro Virus Infections:** Specially prepared ribavirin derivatives AVS5054, 5055, 5056 and 5057 were evaluated against i.c.-induced Balliet PTV infections in 4 week-old female Swiss Webster mice by inoculating the maximum tolerated doses (MTD), the MTD/2, MTD/4 and MTD/8 in DMSO of each i.v. 4 hr pre- or 24 hr post-virus inoculation. AVS5054 and AVS5056 pretreatment resulted in moderately significant increases in mean survival time of the infected mice. AVS5055 pre-treatment resulted in a significant increase in survivors at the MTD of the compound. AVS5057 was not effective. None of these compounds, nor AVS01 in saline, were effective when administered after virus inoculation. **Induction of Lethal Disease in Mice Using Intranasal Administration of Balliet Strain Punta Toro Virus:** Intranasal administration of Balliet strain PTV was lethally infective to 3 week-old C57BL/6 mice, although the LD50 was only a $10^{-0.5}$ dilution of the stock virus. The deaths occurring were accompanied by signs of CNS effects. Virus titers in the brains reached maximal levels by day 10 after initial virus exposure. This peripheral virus inoculation technique may result in a less challenging infection for evaluation of antiviral compounds. Intraperitoneal injection of PTV in either 3 or 4 week-old mice was not acceptably lethal. **Punta Toro Virus-Induced Hematologic Effects in C57BL/6 Mice:** Infection of C57BL/6 mice with Adames PTV results in a rapid and profound suppression of WBC, lymphocytes, T cells and suppressor/cytotoxic T cells by 2 days after virus inoculation. **Effects of Various Metabolic Precursors on the Anti-Punta Toro Virus Activity of AVS206:** The *in vitro* anti-PTV activity of AVS206 was reversed by adenosine, 2-deoxyadenosine, guanosine, guanosine 5'-PO_4, and 2-deoxyguanosine. Other precursors, including inosine, adenine, cytidine, thymidine, uridine, and xanthosine did not have a reversal effect. The anti-PTV activity of AVS01 was reversed by guanosine but not by xanthosine. **Effects of AVS01 and AVS206 on Uptake of Radiolabeled Metabolic Precursors as a Measure of Cytotoxicity:** Neither AVS206 nor AVS01 were considered strongly cytotoxic as measured by effects on DNA, RNA, and protein synthesis. AVS206 was less inhibitory to DNA synthesis as measured by uptake of ^{32}P than AVS01. This suggests both materials to have a static, rather than toxic, effect on LLC-MK_2 cells. **Investigations into the Deamination of AVS206:** AVS206 and AVS01 were subjected to enzymatic degradation with adenosine deaminase. AVS206 was broken down to a species that comigrated with AVS01 as determined by silica gel thin layer chromatography. AVS01 was unaffected. With prolonged incubation, AVS206 degenerated to a species that also comigrates with AVS01. **Interferon Induction by AVS2776:** AVS2776 was a potent and rapid inducer of interferon (IFN) at doses of 100-400 mg/kg given in a single p.o. treatment to C57BL/6 mice. The IFN levels were maximal at 2 hr after treatment. A dose of 50 mg/kg did not induce acceptable levels of IFN. **Immunological Effects of AVS2149 in C57BL/6 Mice:** Single treatment i.p. of C57BL/6 mice with 1, 3.2, or 10 mg/kg of AVS2149 resulted in an increase in splenic B cells and T_S cells and an increase in T cells in the thymus. B and T cell function and macrophage function (IL-1 activity) decreased in these animals. **Effects of Diluent on *In Vitro* and *In Vivo* Infectivity of Punta Toro Virus:** The PTV used in our chemotherapy experiments was more infectious when diluted in tissue culture medium with FBS than in PBSS. The virus was quite sensitive to acid pH and to warm temperature. An animal titration using PTV diluted in the medium and kept cold indicated the virus to be lethally infective with no "window" of infection as seen previously using PBSS as diluent. **Studies to determine the *In Vitro* Antiviral Activity of Immunomodulators AVS2149 and AVS1761:** Treatment of LLC-MK_2 derivative cells with AVS1761 or 2149 up to 4 hr prior to PTV infection did not protect the cells if the compounds were removed prior to virus exposure. Treatment with either compound prior to and continued during virus exposure provided some protection. The protection was proportional to the pre-virus treatment time. **Presentations and Publications:** A total of 10 presentations have been made or have been submitted as abstracts for scientific meetings this year. Three papers have been submitted and accepted to scientific journals for publication.

SUMMARY

1. Preliminary *In Vivo* Assessment of Toxicity: Approximate LD50 values were obtained in 3-4 week-old C57BL/6 mice for 53 AVS compounds.

2. *In Vitro* Evaluation of Test Compounds Against Adames Strain Punta Toro Virus: A total of 305 AVS compounds were evaluated one or more times against the Adames strain of Punta Toro virus (PTV) in LLC-MK2 cells. The PTV-inhibitory compounds were AVS01 (run as a positive control), 206, 347, 361, 2543, 2563, 2714, 2811, 2812, 2869, 2980, 3038, 3593, 3705, 3910, 3982, 4071, 4074, 4113, 4116, 4200, 4592, and 4617.

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removed prior to virus exposure. Treatment of the cells with either compound prior to and continued during virus exposure provided some protection against virus. The protection was proportional to the pre-virus treatment time.

23. Studies to Determine the Nature of the Interference of Infectivity of Punta Toro Virus: Interference to PTV lethality seen at high doses of virus when PTV preparations were titered in mice was found to be due to the virus diluent used. There seems to be sufficient virus inactivated by PBSS to cause significant interference of the mortality one would expect at low dilutions of PTV pools. Use of MEM with FBS can eliminate such interference.

24. Presentations and Publications: A total of 10 presentations have been made or have been submitted as abstracts for scientific meeting this year. Three papers have been submitted and accepted to scientific journals for publication.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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I. PRELIMINARY *IN VIVO* ASSESSMENT OF TOXICITY

Introduction

Before compounds submitted to us can be evaluated for *in vivo* PTV activity, information is needed regarding the approximate LD50 of those compounds as determined using the same treatment schedule to be used in the antiviral experiments. This report summarizes the LD50 data generated either from preliminary toxicity assessment experiments or from data derived from use of concomitantly run toxicity controls in actual PTV experiments. Since some compounds submitted to us are immune modulating materials their most effective dose is often remote from the maximum tolerated dose. In such cases, we are usually instructed by USAMRIID personnel on the doses to use in *in vivo* PTV experiments and we seldom have a need to determine an LD50 dose. Some data regarding these immunomodulating compounds is also included in this section, however, to provide information for others desiring to use such compounds.

Materials and Methods

Compounds: All compounds were submitted to us by Technassociates, Inc. (Rockville, MD). The compounds were weighed and dissolved or suspended in vehicles considered most appropriate for the compound. These vehicles were physiological saline for injection, sterile water for injection, or 4% carboxy methylcellulose in physiological saline.

Animals: C57BL/6 mice 3-4 weeks of age were obtained rom Simonsen Laboratories (Gilroy, CA). All were quarantined at least 24 hr prior to use, and maintained on Wayne Lab Blox mouse chow and tap water ad libitum. All were caged in shoe box style polycarbonate cages with Sani-cell bedding used. All were housed 5 to a cage.

Toxicity Assessments: Mice were injected with varying 2-fold dilutions of each compound according to the indicated treatment regimens. All were weighed immediately prior to treatment and again 18 hr after the final treatment to determine if normal weight gain occurred. In preliminary toxicity studies, the mice were held a total of 14 days. When used as parallel toxicity controls in PTV studies, the animals were held a total of 21 days. Five mice were used at each dosage level. The volume administered was 0.01 ml/g of body weight. Parameters for evaluation included weight change, obvious signs of distress such as diarrhea, prostration, or tremors, and death which was noted daily. The LD50 dose was calculated by the Reed-Muench method (1).

Results and Discussion

The toxicity determinations, expressed as approximate LD50 values, are summarized in Table I-1. Data on 53 compounds are shown. In some cases ">" values are shown because we as yet have not achieved a lethal dose. Values shown as "~" were estimated based on the observation that slightly lower doses were lethal, but to less than 50% of the animals, or treatment with the lower dose caused marked weight loss in the animals, suggesting the MTD dose had essentially been reached.

Conclusions

Approximate LD50 values were obtained in 3-4 week-old C57BL/6 mice for 53 AVS compounds.

References

1. Reed, L.J. and H. Muench. (1938) A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27:493-497.

Table I-1. Preliminary Toxicity Evaluations of AVS Compounds[a]

Compound (AVS No.)	Name	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
01	Ribavirin	bid x 5	s.c.	150
02	Ribavirin 2',3',5'-triacetate	bid x 5	p.o.	450
52	Thioformycin B	once only	s.c.	~2000
		tid x 5	p.o.	~800
79	9-ß-D-Ribofuranosylpurine-6-thiocarboxamide	once only	p.o.	~900
111	Tiazofurin	once only	s.c.	~3000
		bid x 5	p.o.	>750
147	Enviroxime	once only	p.o.	~1500
215	3-Dazaguanosine	qd x 5	s.c.	~100
222	3-Bromo-4-chloropyrazolo[4,4-d]-pyrimidine	bid x 5	s.c.	~3000
		tid x 5	s.c.	>500
		once only	i.p.	~1800
		bid x 5	s.c.	~2200
233	Formycin	bid x 5	s.c.	~900
		once only	s.c.	~3600
257	Tiazofurin 5'-monophosphate	bid x 5	i.p.	>400
272	3-Deazaguanine	qd x 5	s.c.	~600
360	7-Deoxynarciclasine	bid x 5	s.c.	>500
1761	Poly IC•LC	qd x 8	i.p.	~8
1767	AM-3	once only	s.c..	~2000
		bid x 5	i.p.	~800
1778	Mannozym	once only	s.c.	~100
		bid x 5	i.p.	200
1969	CL259,763	once only	p.o.	>200
		once only	i.p.	>200
		bid x 5	i.p.	>100
		bid x 5	p.o.	>200
1976	Thymine riboside 2',3'-dialdehyde	bid x 5	i.p.	>100
		once only	i.p.	375
2149	Ampligen	once only	i.p.	~20
2700	6-Ethyl thiopurine riboside	bid x 5	i.p.	300
2712	Bryostatin 1	qd x 5	i.p.	>144 µg/kg/day
		once only	i.p.	>200 µg/kg
2713	Bryostatin 2	qd x 5	i.p.	>36 µg/kg/day
2741	1-(ß-D-ribofuranosyl)-1,2,4-triazole-3-(1,4,5,6-tetrahydropyrimidine)•HCl	bid x 5	s.c.	>2000

Compound (AVS No.)	Name	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
2742	1-(ß-D-ribofuranosyl)-1,2,4-triazole-3-(5-hydroxy-1,4,5,6-tetrahydro-pyrimidine•HCl	bid x 5	s.c.	>2000
2776	2-Amino-5-bromo-6-phenyl-4(3H)-pyrimidinone (ABPP)	qd x 3	p.o.	~800
		once only	p.o.	>400
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	qd x 3	p.o.	>400
2778	2-Amino-5-bromo-6-methyl-4(3H)pyrimidinone (ABMP)	qd x 3	p.o.	>400
		once only	p.o.	>400
2779	MVE-1	once only	i.p.	>100
2880	Oxamisole	qd x 2	p.o.	>50
2933	CGP19835 (Liposome monomer tripeptide	once only	i.p.	>1000
		once only	i.p.	>10,000
		eod x 3	i.p.	>1,000
2978	Tetrahydroxy tetraacetate of 7-deoxynarciclasine	bid x 7	i.p.	>400
2980	Tetrahydroxy analog of pancratistatin	once only	i.p.	~100
		bid x 5	i.p.	~15
3425	8-Bromoguanosine	bid x 5	i.p.	550
		once only	s.c.	~500
		qd x 5	s.c.	~350
3580	Unidentified	bid x 5	i.p.	>100
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	once only	p.o.	>500
3588	Meta fluoro derivative of ABPP	once only	i.p.	~600
3589	2-Amino-5-chloro-2,3-difluorophenyl-4(3H)-pyrimidinone	once only	p.o.	>500
3593	LY253,963	tid x 5	i.p.	>150
		bid x 6	i.p.	>150
		once only	i.p.	>500
		ad lib	p.o. (drink water)	>93
3706	Tiazofurin triacetate	bid x 5	s.c.	>2000
		bid x 5	s.c.	~3000
3925	du Pont A2222-1	qd x 5	i.p.	19
		once only	i.p.	150
		eod x 3	i.p.	>40
3926	du Pont A2227-1	once only	i.p.	75
		bid x 5	i.p.	>40
		once only	i.p.	200
3927	du Pont A754-1	once only	i.p.	180
		bid x 5	i.p.	>40
		once only	i.p.	~400
3933	Germanium 089	qd x 5	i.p.	>250

Compound (AVS No.)	Name	Treatment Schedule	Treatment Route	Approximate LD50 (mg/kg/day)
3934	Ge132	qd x 7	i.p.	>1000
		bid x 7	p.o.	>600
		bid x 7	i.p.	>300
3960	DMG	bid x 5	s.c.	>900
		bid x 7	i.p.	>600
		bid x 7	p.o.	>800
4113	Pseudolycorine•HCl	qd x 5	s.c.	>12
4282	AM-5	once only	i.p.	~2
4283	AM-6	once only	i.p.	>200
4284	AM-7	once only	i.p.	>180
4285	AM-8	once only	i.p.	>100
4286	P-136	once only	i.p.	>200
4287	P-117	once only	i.p.	>200
4593	P-188	once only	i.p.	>200
4616	Noxymethyl pennicillinic acid	bid x 5	s.c.	>150

[a]3-4 week-old C57BL/6 mice.

II. *IN VITRO* EVALUATION OF TEST COMPOUNDS AGAINST ADAMES STRAIN PUNTA TORO VIRUS

Introduction

The initial phase of our anti-PTV testing program is the in vitro evaluation of compounds submitted to us. In the initial tests, inhibition of cytopathic effect (CPE) is determined against the Adames strain of PTV. Compounds exhibiting adequate CPE inhibition (Virus Rating [VR] ≥ 0.5) are retested and their effects on virus yield (Virus Titer Reduction [VTR]) at the maximum tolerated dose are also determined.

This section describes our experiments with 305 AVS compounds evaluated during this report period.

Materials and Methods

Virus: A twice plaque isolated PTV, both Adames and Balliet strains, prepared in LLC-MK_2 cells as described in Section I of our First Annual Report was used.

Cells: LLC-MK_2 (Rhesus monkey kidney) cells were used. They were obtained initially from the ATCC. Various passages of the cells were used over the 1-year period of this study. Growth medium was medium essential medium (MEM, GIBCO Labs, Grand Island, NY) containing 5% fetal bovine serum (FBS, HyClone Labs, Logan, UT) and 0.1% $NaHCO_3$ without antibiotics. All were determined to be mycoplasma-free.

Test Compounds: All materials were provided by Technassociates for these tests. Each was stored and handled according to instructions from Technassociates.

In Vitro Testing Procedures: Seven concentrations of test compound, these concentrations usually being 1000, 320, 100, 32, 10, 3.2 and 1 µg/ml, were added in 0.1 ml amounts to an 18-hr monolayer of cells in 96-well flat-bottom microplates. Adames strain PTV (320 CCID50/ml) was added in 0.1 ml volume 15 minutes later. Three virus-containing cups in each microplate were used for each compound dosage level, with one cup used for toxicity controls (cells + sterile virus diluent + compound). Six cups in each panel were used for virus controls (cells + virus + drug diluent) and 6 cups in each panel were used for normal cell controls (cells + sterile virus diluent + drug diluent). Test medium in which virus and compound were suspended or dissolved was MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg/ml gentamicin. Viral CPE was graded from 0 (normal cells) to 4 (virtually complete destruction of the cell layer) 6-7 days post-virus exposure. The CPE was read by an individual who was well trained for CPE evaluation, then this reading was confirmed by a second, similarly trained individual.

Reduction in CPE was evaluated by VR as we have described previously (1, 2) and by 50% effective dose (ED50). The VR is a numerical expression of antiviral activity, taking into account percent of CPE inhibition and partial cytotoxicity of the test compound. In our experience a VR of 1.0 is indicative of definite antiviral activity, a VR of 0.5 - 0.9 indicates moderate activity, and a VR of <0.5 suggests slight activity perhaps resulting from cytotoxicity only. The ED50 was determined by plotting percent CPE inhibition vs test compound concentration, with the ED50 level being that level causing an approximate 50% CPE inhibition. Also included in the test was an estimated maximum tolerated dose (MTD) of the test compound, this MTD being the lowest dosage causing visually discernible cytotoxic effects in the concurrently run toxicity controls. Cytotoxicity was determined by microscopic assessment of compound-induced cytopathic effects in treated cultures compared with those in control cells run in the same plate.

Validation of apparent positive activity was done in early *in vitro* experiments by fixing the drained cells in 10% formalin and staining them with 1% crystal violet, which clearly demonstrated the complete cell monolayer. The stained plate was labeled and photographed. The experiment was then repeated and, in addition to CPE inhibition being determined, virus yield was also determined by freezing the plate, thawing at room temperature, and the medium from each 10-fold dilution and from virus controls removed and virus quantified. The virus quantification was done by end-point dilution, determining CPE induced in triplicate cups containing LLC-MK_2 cells exposed to 0.1 ml of 10-fold dilutions of each sample collected.

As positive control, ribavirin (AVS01) was tested in parallel in each series of tests. This compound was shown by us (Section I, First Annual Report) and (3) to be highly active vs PTV *in vitro* and *in vivo*.

Compounds exhibiting confirmed positive activity in these tests were retested in a similar manner using the Balliet strain of virus. These latter experiments are described in Section III of this report.

Results and Discussion

The results of all *in vitro* experiments run against the Adames PTV are summarized in Table II-1. A total of 305 compounds were tested, and 22 were considered positive against the virus. The PTV-inhibitory materials were AVS01 (run as a positive control), 206, 347, 361, 2543, 2563, 2714, 2811, 2812, 2869, 2980, 3038, 3593, 3705, 3910 (data not confirmed), 3982, 4071, 4074, 4113, 4116, 4200, 4592, and 4617.

Conclusions

A total of 305 AVS compounds were evaluated one or more times against the Adames strain of Punta Toro virus (PTV) in LLC-MK2 cells. The PTV-inhibitory compounds were AVS01 (run as a positive control), 206, 347, 361, 2543, 2563, 2714, 2811, 2812, 2869, 2980, 3038, 3593, 3705, 3910, 3982, 4071, 4074, 4113, 4116, 4200, 4592, and 4617.

Table II-1. Summary of *In Vitro* Anti-Punta Toro Virus (Adames Strain) Activity of AVS Compounds.

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
000001	0.8-1.4	2.2-17	10-32	-	1.1-8.0	S
000206	0.8, 0.7	20, 51	32, 10	-	1.6, 0.2	S
000303	0.3	160	1	-	0.01	S
000346	0.0	>1000	1000	-	<1.0	INS
000347	0.5, 0.8	6, 6	1.0, 1.0	0.2	0.2, 0.2	INS
000361	0.8, 0.6	2, 2	1.0, 1.0	0.5	0.5, 0.5	INS
002543	0.7	30	1.0	0.5	0.03	S
002563	0.8, 0.6	5, 7.5	10, 1.0	-	2, 0.1	INS
002712	0.4*	2*	0.32*	-	0.16	S
002713	0.0	>10	10	-	<1.0	S
002714	0.6	10	<1.0	0.0	0.1	INS
002811	0.7	3.0	<0.32	0.1	0.1	INS
002812	0.4-0.6	0.25-1.0	<1.0-0.1	-	<1.0-0.4	INS
002869	0.5	150	320	0.3	2.1	S
002933	0.0	>1000	1000	-	<1.0	S
002979	0.4	90	32	-	0.4	INS
002980	0.8	8.6	3.2	0.1	0.4	INS
003038	1.2	0.096	0.01	0.2	0.1	S
003040	0.0	>320	320	-	0.1	S
003041	0.0	>1000	>1000	-	<1.0	INS
003042	0.0	>100	100	-	<1.0	INS
003043	0.2	820	100	-	0.1	INS
003245	0.4	160	100	-	0.6	S
003425	0.0	>1000	>1000	-	<1.0	INS
003520	0.1	7.7	1.0	-	0.1	S
003521	0.1	>320	320	-	<1.0	S
003522	0.1	>1000	1000	-	<1.0	S
003523	0.0	>100	100	-	<1.0	S
003524	0.0	>1000	320	-	<1.0	S
003525	0.0	>1000	320	-	<1.0	S
003526	0.0	>1000	1000	-	<1.0	INS
003527	0.0	>1000	320	-	<1.0	INS
003528	0.2	300	320	-	1.1	INS
003529	0.3	160	10	-	0.06	S
003530	0.0	>1000	1000	-	<1.0	S

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
003531	0.1	640	100	-	0.2	S
003532	0.2	640	100	-	0.2	S
003533	0.0	>1000	1000	-	<1.0	S
003534	0.0	>1000	1000	-	<1.0	S
003535	0.0	>1000	100	-	<1.0	S
003536	0.0	>1000	1000	-	<1.0	S
003537	0.2	75	10	-	0.1	INS
003538	0.0	>1000	1000	-	<1.0	INS
003539	0.1	32	10	-	0.3	S
003540	0.1	>1000	320	-	0.3	S
003541	0.1	320	100	-	0.3	INS
003542	0.0	>32	10	-	<1.0	S
003543	0.0	>1.0	1.0	-	<1.0	S
003544	0.0	>1000	1000	-	<1.0	S
003545	0.4	320	1000	-	3.1	S
003546	0.0	>1000	320	-	<1.0	INS
003547	0.0	>1000	1000	-	<1.0	INS
003548	0.0	>1000	320	-	<1.0	INS
003549	0.0	>1000	320	-	<1.0	INS
003550	0.1	850	32	-	0.04	S
003551	0.0	>1000	1000	-	<1.0	INS
003552	0.0	>1000	320	-	<1.0	S
003553	0.1	88	32	-	0.4	S
003554	0.1	640	100	-	0.2	S
003555	0.1	1000	1000	-	1.0	S
003556	0.1	96	32	-	0.3	S
003557	0.2	220	100	-	0.5	INS
003558	0.2	600	320	-	0.5	INS
003559	0.1	1000	320	-	0.3	INS
003560	0.0	>320	320	-	<0.1	INS
003561	0.0	>1000	320	-	<0.1	INS
003562	0.0	>100	100	-	<0.1	INS
003563	0.0	>1000	1000	-	<0.1	S
003572	0.0	>100	32	-	<0.1	S
003573	0.1	32	32	-	1.0	S
003574	0.0	>320	320	-	<1.0	INS
003575	0.1	32	10	-	0.3	S
003576	0.1	>1000	100	-	<1.0	S
003577	0.0	>1000	320	-	<1.0	S

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
003578	0.0	>1000	1000	-	<1.0	S
003579	0.0	>100	10	-	<1.0	S
003580	0.0	>100	3.2	-	<1.0	S
003581	0.0	>100	100	-	<1.0	S
003582	0.0	>1000	320	-	<1.0	S
003583	0.0	>320	320	-	<1.0	S
003584	0.0	>320	320	-	<1.0	S
003585	0.0	>1000	>1000	-	<1.0	S
003587	0.0	>1000	32	-	<1.0	S
003593	0.6-1.2,	0.9->1000	<0.1-0.32	0.5	<0.01-<0.1,	S
003602	0.2	640	1000	-	1.6	S
003603	0.0	>1000	10	-	<1.0	INS
003605	0.0	>1000	100	-	<1.0	S
003606	0.0	>1000	100	-	<1.0	S
003607	0.0	>1000	1000	-	<1.0	S
003608	0.1	240	100	-	0.4	INS
003609	0.0	>1000	320	-	<1.0	INS
003610	0.1	375	10	-	0.03	S
003611	0.0	>1000	1000	-	<1.0	INS
003612	0.3	215	100	-	0.5	S
003613	0.0	>1000	320	-	<1.0	INS
003614	0.1	>1000	320	-	0.3	INS
003615	0.0	>1000	1000	-	<1.0	INS
003625	0.1	32	3.2	-	0.1	INS
003677	0.0	>1000	1000	-	<1.0	S
003678	0.0	>10	10	-	<1.0	S
003679	0.3	18	32	-	1.8	INS
003680	0.0	>1000	320	-	<1.0	S
003703	0.1	351	100	-	0.3	INS
003704	0.0	>320	320	-	<1.0	INS
003705	1.1, 0.9	3.5, 2.2	1, 1	ot[g]	0.3, 0.5	S
003706	0.4	110	10	-	0.09	S
003707	0.0	>1000	1000	-	<1.0	INS
003708	0.0	>100	320	-	<1.0	INS
003709	0.0	>100	100	-	<1.0	INS
003710	0.0	>320	320	-	<1.0	INS
003711	0.0, 0.0	>320, >320	100, 320	-	<1.0, <1.0	S
003712	0.0, 0.0	>1000, >1000	320, 1000	-	<1.0, <1.0	INS
003713	0.1	1000	320	-	0.3	INS

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
003714	0.0	>1000	1000	-	<1.0	INS
003715	0.0	>1000	320	-	<1.0	INS
003716	0.0	>1000	320	-	<1.0	INS
003717	0.1, 0.1	320, >1000	100, 1000	-	0.3, <1.0	S
003721	0.0	>10	10	-	<1.0	S
003722	0.2	1000	1000	-	1.0	S
003723	0.0	>1000	>1000	-	<1.0	INS
003724	0.2	562	1000	-	1.8	S
003725	0.1	90	3.2	-	0.04	INS
003726	0.1	268	32	-	0.1	INS
003906	0.0, 0.0	>1000, >1000	100, 1	-	<1.0, <1.0	S
003907	0.0, 0.0	>1000, >1000	320, 1000	-	<1.0, <1.0	S
003908	0.4, 0.2	560, 557	<1.0, 1000	-	0.002, 1.8	S
003909	0.1	>1000	1000	-	<1.0	S
003910	0.6, 0.0	1000, >1000	?, 1000	-	?, <1.0	S
003911	0.2	446	1000	-	2.2	S
003912	0.1	1000	10	-	0.01	S
003913	0.2	~1000	1000	-	~1.0	S
003914	0.3	356	320	-	0.9	S
003915	0.2	548	320	-	0.6	S
003916	0.2	~7000	1000	-	0.14	S
003917	0.3	36	10	-	0.3	S
003918	0.0	>1000	1000	-	<1.0	INS
003919	0.2	861	320	-	0.4	INS
003920	0.3, 0.0	292, >1000	1000, ≤1.0	-	3.4, <1.0	S
003921	0.0	1000	>1000	-	<1.0	S
003922	0.3	294	1000	-	3.4	S
003923	0.1	><1000	<320	-	<1.0	INS
003924	0.0	>1000	1000	-	<1.0	S
003925	0.0	>1000	1000	-	<1.0	INS
003926	0.0	>100	100	-	<1.0	INS
003927	0.2	160	10	-	0.06	INS
003933	0.0	>320	320	-	<1.0	INS
003935	0.0	>1000	10	-	<1.0	S
003936	0.2	385	320	-	0.8	S
003937	0.0	>1000	1000	-	<1.0	S
003938	0.0	>1000	>1000	-	<1.0	S
003939	0.1	>320	100	-	<1.0	S
003941	0.0	>1000	3.2	-	<1.0	S

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
003942	0.0	>1000	320	-	<1.0	S
003943	0.0	>1000	1000	-	<1.0	S
003944	0.0	>320	100	-	<1.0	S
003945	0.1	>1000	1000	-	<1.0	INS
003946	0.1	>1000	>1000	-	<1.0	S
003947	0.0	>1000	>1000	-	<1.0	S
003960	0.0	>1000	320	-	<1.0	S
003966	0.0	100	32	-	0.3	S
003980	0.0	32	1	-	0.03	INS
003981	0.1	>1000	1000	-	<1.0	S
003982	0.5, 0.6	97, 60	320, 320	-	3.3, 5.3	S
003983	0.0	>1000	1000	-	<1.0	S
003984	0.1	759	1000	-	1.3	S
003985	0.2	449	1000	-	2.2	S
003986	0.2	415	320	-	0.8	S
003987	0.2	68	100	-	1.5	S
003989	0.0	>1000	>1000	-	<1.0	INS
003990	0.2	681	1000	-	1.5	INS
003991	0.0	>1000	>1000	-	<1.0	INS
003992	0.0	>1000	1000	-	<1.0	INS
003993	0.0	>1000	1000	-	<1.0	S
003994	0.0	>1000	>1000	-	<1.0	INS
003995	0.0	>1000	>1000	-	<1.0	INS
003996	0.1	794	1000	-	1.3	INS
003997	0.0	>1000	>1000	-	<1.0	INS
003998	0.0	>1000	320	-	<1.0	INS
003999	0.0	>1000	320	-	<1.0	INS
004000	0.0	>320	32	-	<0.1	INS
004001	0.0	>1000	1000	-	<1.0	S
004002	0.0	>1000	320	-	<0.3	S
004003	0.0	>1000	1000	-	<1.0	INS
004004	0.0	>1000	1000	-	<1.0	S
004005	0.2	173	100	-	0.6	INS
004006	0.2	193	320	-	1.7	INS
004042	0.1	>1000	320	-	<0.3	S
004043	0.1	>1000	1000	-	<1.0	S
004044	0.1	320	100	-	0.3	S
004045	0.1	>1000	320	-	<0.3	S
004046	0.0	>320	320	-	<1.0	S

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
004047	0.1	26	3.2	-	0.1	INS
004048	0.0	>1000	1000	-	<1.0	INS
004049	0.0	>100	100	-	<1.0	INS
004051	0.2	2	<1	-	0.5	S
004053	0.0	>320	100	-	<0.3	S
004064	0.0	>1000	1000	-	<1.0	INS
004065	0.0	>1000	<1	-	<1.0	S
004068	0.0	>1000	32	-	<0.03	INS
004070	0.1	1000	320	-	0.3	INS
004071	0.6-0.9	34-180	32-320	2.8	0.2-9.4	S
004072	0.0	>32	10	-	<1.0	S
004073	0.1	1000	320	-	0.3	INS
004074	0.9, 0.6	1, 1.2	0.01, 3.2	ot	0.01, 2.7	S
004094	0.2	141	100	-	0.7	S
004095	0.0	>1000	320	-	<0.3	INS
004103	0.0	>1000	1000	-	<1.0	S
004104	0.0	>1000	320	-	<0.3	S
004105	0.0	>1000	>1000	-	<1.0	INS
004108	0.0	>320	320	-	<1.0	S
004109	0.0	>1000	>1000	-	<1.0	S
004110	0.2	190	320	-	1.7	S
004111	0.4-0.6	133-377	320-1000	0.3	2.4-5.1	S
004112	0.2	410	320	-	0.8	S
004113	0.8, 0.9	1, 0.8	0.01, 3.2	ot	0.01, 4.0	S
004114	0.2	750	100	-	0.1	S
004115	0.4	150	96.2	-	0.6	S
004116	0.3-0.5	170-649	320-1000	-	0.6-1.9	S
004117	0.0	>1000	>1000	-	<1.0	S
004118	0.0	>1000	1000	-	<1.0	S
004119	0.0	>1000	100	-	<0.1	S
004123	0.0	>1000	320	-	<0.1	S
004124	0.0	>320	320	-	<0.1	INS
004125	0.2	407	320	-	0.8	S
004126	0.2	63	32	-	0.5	INS
004127	0.0	>100	32	-	<0.1	INS
004128	0.0	>1000	>1000	-	<0.1	S
004129	0.0	>1000	32	-	<0.1	S
004130	0.0	>32	3.2	-	<0.1	S
004131	0.0	>1000	>1000	-	<0.1	INS

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
004135	0.0	>1000	>1000	-	<0.1	S
004136	0.0	>1000	>1000	-	<0.1	S
004137	0.0	>1000	1000	-	<0.1	S
004138	0.0	>1000	1000	-	<0.1	S
004139	0.1	>1000	100	-	<0.1	S
004140	0.1	>100	320	-	<0.1	S
004141	0.0	>1000	1000	-	<0.1	S
004149	0.0	>1000	1000	-	<0.1	S
004150	0.2	>1000	1000	-	<0.1	S
004152	0.0	>100	320	-	<0.1	INS
004153	0.0	>1000	>1000	-	<0.1	INS
004154	0.0	>1000	>1000	-	<0.1	INS
004155	0.0	>100	100	-	<0.1	INS
004156	0.1	1000	320	-	0.32	S
004162	0.0	>1000	>1000	-	<0.1	S
004163	0.0	>1000	>1000	-	<0.1	S
004166	0.0	>1000	>1000	-	<0.1	S
004167	0.3	1000	3.2	-	0.003	S
004168	0.0	>1000	>1000	-	<0.1	S
004173	0.0	>1000	1000	-	<0.1	INS
004174	0.0	>1000	320	-	<0.1	S
004175	0.0	>1000	>1000	-	<0.1	INS
004176	0.0	>1000	1000	-	<0.1	INS
004177	0.0	>1000	>1000	-	<0.1	INS
004178	0.0	>1000	1000	-	<0.1	S
004179	0.2	562	1000	-	1.8	S
004180	0.2	562	320	-	0.6	S
004181	0.0	>320	320	-	<0.1	S
004182	0.0	>1000	320	-	<0.1	INS
004183	0.0	>1000	1000	-	<0.1	INS
004184	0.0	>1000	320	-	<0.1	INS
004185	0.0	>1000	1000	-	<0.1	INS
004186	0.0	>320	320	-	<0.1	INS
004187	0.0	>100	100	-	<0.1	S
004188	0.0	>32	32	-	<0.1	S
004189	0.1	>320	100	-	0.3	INS
004190	0.0	>1000	320	-	<0.1	INS
004191	0.0	>1000	320	-	<0.1	INS
004192	0.0	>1000	320	-	<0.1	INS

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
004193	0.0	>1000	1000	-	<0.1	INS
004194	0.0	>1000	>1000	-	<0.1	INS
004195	0.0	>320	320	-	<0.1	S
004196	0.0	>1000	320	-	<0.1	S
004197	0.1	>320	32	-	<0.1	INS
004198	0.0	>100	32	-	<0.1	INS
004199	0.0	>1000	1000	-	<0.1	INS
004200	0.5	1000	320	-	0.3	INS
004201	0.0	>1000	1000	-	<0.1	S
004202	0.0	>1000	1000	-	<0.1	INS
004203	0.0	>1000	1000	-	<0.1	INS
004204	0.1	749	320	-	0.4	S
004205	0.0	>1000	>1000	-	<0.1	INS
004206	0.0	>1000	>1000	-	<0.1	S
004207	0.0	>1000	320	-	<0.1	S
004208	0.1	>320	100	-	<0.1	S
004209	0.0	>1000	320	-	<0.1	S
004211	0.0	>1000	1000	-	<0.1	S
004213	0.0	>1000	1000	-	<0.1	S
004214	0.0	>1000	>1000	-	<0.1	INS
004215	0.0	>1000	320	-	<0.1	S
004216	0.1	>1000	320	-	<0.1	INS
004217	0.0	>1000	>1000	-	<0.1	INS
004218	0.0	>1000	320	-	<0.1	INS
004219	0.1	>1000	100	-	<0.1	INS
004220	0.0	>1000	320	-	<0.1	INS
004221	0.0	>1000	320	-	<0.1	INS
004222	0.0	>1000	320	-	<1.0	S
004224	0.0	>320	32	-	<0.1	S
004225	0.0	>320	32	-	<0.1	S
004226	0.0	>1000	100	-	<0.1	S
004227	0.0	>320	10	-	<0.1	S
004228	0.0	>100	32	-	<0.1	INS
004229	0.0	<32	<10	-	<0.1	INS
004230	0.0	>100	<100	-	<0.1	INS
004231	0.0	>1000	>1000	-	<0.1	S
004232	0.0	>1000	<1000	-	<0.1	INS
004233	0.0	>100	320	-	<0.1	S
004235	0.6	54	10	-	0.2	S

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR[d] at MTD (log_{10})	TI[e]	Aqueous Solubility[f]
004239	0.0	>10	10	-	<0.1	S
004240	0.3	4	≥1.0	-	≥0.25	S
004241	0.2	18	10	-	0.6	S
004242	0.0	>1000	<320	-	<0.1	INS
004243	0.0	>320	320	-	<0.1	S
004244	0.2	<56	<32	-	<0.1	INS
004245	0.0	>1000	<1000	-	<0.1	INS
004246	0.0	>1000	<320	-	<0.1	INS
004247	0.2	>320	<32	-	<0.1	INS
004248	0.1	>320	<10	-	<0.1	INS
004249	0.0	>1000	<320	-	<0.1	INS
004250	0.0	>1000	>1000	-	<0.1	INS
004251	0.1	>1000	<1000	-	<0.1	INS
004252	0.2	610	32	-	0.1	INS
004253	0.0	>32	1.0	-	<0.1	S
004254	0.1	365	32	-	0.1	INS
004255	0.0	>320	10	-	<0.1	INS
004256	0.0	>1000	320	-	<0.1	INS
004257	0.0	>1000	1000	-	<0.1	INS
004258	0.2	173	10	-	0.1	INS
004259	0.1	10	1.0	-	0.1	S
004285	0.0	>1000	320	-	<0.1	INS
004592	0.6-0.7	13-75	10-100	0.2	0.8-1	S
004617	0.4-0.6	153-272	320-1000	0.5	1.3-3.7	S

*Compound was dissolved in methanol; methanol used alone exhibited essentially the same antiviral effect.

[a]Virus Rating.

[b]50% Effective Dose.

[c]Minimum Toxic Dose.

[d]Virus Titer Reduction as determined at the MTD.

[e]Therapeutic Index (MTD÷ ED50).

[f]S: Soluble; INS: Moderately Insoluble; VINS: Very Insoluble.

III. *IN VITRO* EVALUATION OF TEST COMPOUNDS AGAINST BALLIET STRAIN PUNTA TORO VIRUS

Introduction

As a follow-up to the active leads found in the initial *in vitro* anti-PTV testing described in Section II, active compounds are then evaluated against the Balliet strain of PTV. The results of this phase of *in vitro* testing is described here.

Materials and Methods

Virus: A twice plaque isolated PTV, Balliet strain, obtained from the American Type Culture Collection was used. A stock was prepared in LLC-MK_2 cells.

Cells: LLC-MK_2 cells as described in Section II were used.

Test Compounds: All compounds were provided by Technassociates, Inc.

In Vitro Testing Procedures: All testing was done in an identical manner as described in Section II for the Adames virus.

Results and Discussion

A total of 18 compounds were evaluated for in vitro effects against the Balliet PTV. The results are seen in Table II-1. Only AVS3982 was not considered to have activity against this virus. Active materials were AVS01 (used as a positive control), 139, 347, 361, 2563, 2811, 2812, 2980, 3038, 3703, 4071, 4074, 4111, 4113, 4116, 4592, and 4617.

Conclusions

The following AVS compounds were evaluated against the Balliet strain of PTV: AVS01 (used as positive control), 139, 347, 361, 2563, 2811, 2812, 2980, 3038, 3703, 3982, 4071, 4074, 4111, 4113, 4116, 4592, and 4617. Only AVS3982 showed no activity against this virus.

Table III-1. Summary of *In Vitro* Anti-Punta Toro Virus (Balliet Strain) Activity of AVS Compounds.

Compd No. (AVS)	VR[a]	ED50[b] (µg/ml)	MTD[c] (µg/ml)	VTR @ MTD[d] (log_{10})	TI[e]	Aqueous Solubility[f]
000001	0.9-1.2	5.6-8	10-32	-	1.7-5.7	S
000139	0.8	79	3.2	-	0.04	S
000347	0.6, 0.6	9.1, 16	<1.0, 3.2	-	0.1, 0.2	INS
000361	0.6, 0.6	2.2, 6	<1.0, 3.2	-	0.5, 0.5	INS
002563	0.4	16	10	-	0.6	INS
002811	0.8, 0.8	2.0, 2.2	<0.32, <1.0	0.1	0.2, 0.5	INS
002812	0.4	0.6	<0.1	-	0.2	INS
002980	0.6, 0.4	22, 22	32, <3.2	0.0	1.5, 0.2	S
003038	1.8, 1.3	0.08, 0.2	0.01, 0.01	0.1	0.1, 0.1	S
003703	1.1	2.3	1.0	-	0.4	S
003982	0.0	>320	320	-	<0.1	S
004071	0.5	37	100	0.7	2.7	S
004074	0.8, 0.5	2.4, 2.8	≤1, ≤1	-	0.4, 0.4	S
004111	0.4	596	1000	-	1.7	S
004113	0.6-0.8	1.8-2.2	≤1	1.3	0.5-0.6	S
004116	0.7	115	1000	0.2	8.7	S
004592	1.8	-	32	-	-	S
004617	0.7	130	1000	3.0	7.7	S

[a]Virus Rating.

[b]50% Effective Dose.

[c]Minimum Toxic Dose.

[d]Virus Titer Reduction as determined at the MTD.

[e]Therapeutic Index (MTD ÷ ED50).

[f]S: Soluble; INS: Moderately Insoluble.

IV. OVERVIEW OF IN VIVO ANTI-PUNTA TORO VIRUS ACTIVITY OF AVS COMPOUNDS: SUMMARY OF THREE YEARS' TESTING

Introduction

This is the 3rd annual report of anti-Punta Toro virus experiments run by Utah State University (USU) under Contract No. DAMD 17-86-C-6028. It is appropriate to summarize in tabular fashion all the *in vivo* work run to date against this virus. This table is shown in this section. All in vivo experiments, including both Adames and Balliet virus strains, combination studies, and special intravenous therapy studies are seen in Table IV-1.

The following explains the legend for each column in the table:

AVS #: Number assigned to the compound by Technassociates, Inc.

Compound Name: Often an abbreviated name for the compound as provided to us. The short version of the name is used in order to fit it into the space provided.

Expt. #: The USU experiment number (PtA—). Every PTV in vivo experiment is numbered consecutively.

Test Date: The date the experiment was begun.

Treatment Schedule: The schedule used for the animal treatments, indicated in abbreviated form:

bid: Twice daily, usually 8 am and 4 pm
qd: Once daily
tid: Three times daily
single: Once only
qid: Four times daily
eod: Every other day
beg: Beginning, with the hrs indicated pre or post-virus inoculation; if no time is shown, virus was not given to the animals.

Route: Treatment route:
ip: intraperitoneal
sc: subcutaneous
po: oral gavage
ic: intracerebral
iv: intravenous.

Dose Range: Range of doses of the compound used, in mg/kg/day (unless actually shown as µg/kg/day). Doses usually varied by two-fold dilution, although some immunomodulators were used in one-half $\log_{10}$ increments.

Tox. @: The lowest dose (in mg/kg/day or, if indicated, as µg/kg/day) of the compound at which toxicity (death of one or more toxicity control animals) was seen. If a ">" sign is indicated, no toxicity was seen. "All lost weight" indicates the toxicity control mice all lost weight between the time therapy was initiated and 18 hr after treatment was terminated. "ON TEST" indicates the study was not sufficiently complete to indicate actual data at the time the table was prepared.

Results: Our overall impression of the antiviral efficacy seen:
+: Significant ($P<0.05$ or $P<0.01$) increase in survivors.
±: Significant effect on one or more parameters other than survivors (i.e., mean survival time increase; decrease in liver score, SGOT, SGPT, serum virus or liver virus) without a significant survivor increase.
-: No significant effects by any parameter.
TI: Therapeutic index (minimum toxic dose ÷ minimum antivirally effective dose).
?: Designation of a test in which the results were compromised by a poor control result.
ON TEST: Experiment still underway at the time the table was prepared.

MIC: Minimum inhibitory dose, in mg/kg/day or, if indicated in Dose Range column, in µg/kg/day.

Remarks:

EXPANDED: An experiment in which the infection parameters were expanded from survivors/total and mean survival time to include other parameters such as liver score, SGOT, SGPT, serum virus, liver virus, etc.

BALLIET: An experiment run using the Balliet strain of PTV. All other experiments using the Adames strain of PTV.

TI: Therapeutic index determination study.

MMF: Mode modification study (determination of effect of varying virus challenge inoculum concentration).

COMBINATION: An experiment in which a combination of two compounds were evaluated.

REPEAT: An experiment run to repeat a previous unacceptable experiment.

IFN: An experiment run to determine if the compound induced interferon in the animals, and the kinetics of that induction.

IMMUNOLOGY: Experiments in which immunological parameters other than IFN are studied with an immunomodulating compound.

Table IV-1. Overview of In Vivo Anti-Punta Toro Virus Experiments, Dec. 1985-Dec. 1988

AVS#	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
1	Ribavirin	1	7-28-86	bid x 5, beg. 4 hr pre	sc	9.4-75	75	+	9.4	EXPANDED
1	Ribavirin	6	10-16-86	bid x 9, beg 30 hr pre	sc	9.4-75	9.4	-	>75	BALLIET
1	Ribavirin	7	10-16-86	bid x 9, beg 30 hr pre	sc	9.4-75	9.4	-	>75	BALLIET
1	Ribavirin	8	10-23-86	bid x 7, beg 4 hr pre	sc	0.6-75	>75	TI 16	4.7	TI, MMF
1	Ribavirin	9	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	9.4	MMF
1	Ribavirin	10	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	9.4	MMF
1	Ribavirin	11	10-23-86	bid x 7, beg 4 hr pre	sc	9.4-75	>75	+	18.8	MMF
1	Ribavirin	20	1-16-87	bid x 5, beg 24 hr post	sc	37.5-150	150	+	37.5	EXPANDED
1	Ribavirin	21	1-16-87	bid x 5, beg 36 hr post	sc	37.5-150	150	+	37.5	EXPANDED
1	Ribavirin	28	1-22-87	single, beg 4 hr pre	sc	175-700	>700	?		
1	Ribavirin	29	1-22-87	single, beg 8 hr pre	sc	175-700	>700	?		
1	Ribavirin	30	1-22-87	single, beg 24 hr pre	sc	175-700	>700	?		
1	Ribavirin	31	1-22-87	single, beg 48 hr pre	sc	175-700	>700	?		
1	Ribavirin	32	1-22-87	single, beg 72 hr pre	sc	175-700	>700	?		
1	Ribavirin	33	1-22-87	single, beg 96 hr pre	sc	175-700	>700	?		
1	Ribavirin	43	2-5-87	bid x 5, beg 4 hr pre	po	3.2-100	>100	+	12.5	EXPANDED
1	Ribavirin	44	2-5-87	bid x 5, beg 4 hr post	po	3.2-100	>100	+	6.3	EXPANDED
1	Ribavirin	45	2-5-87	bid x 5, beg 24 hr post	po	3.2-100	>100	+	6.3	EXPANDED
1	Ribavirin	46	3-6-87	single, beg 4 hr post	sc	175-700	>700	+	175	
1	Ribavirin	47	3-6-87	single, beg 8 hr post	sc	175-700	>700	+	175	
1	Ribavirin	48	3-6-87	single, beg 24 hr post	sc	175-700	>700	+	175	
1	Ribavirin	49	3-6-87	single, beg 48 hr post	sc	175-700	>700	+	175	
1	Ribavirin	50	3-6-87	single, beg 72 hr post	sc	175-700	>700	±	350	
1	Ribavirin	51	3-6-87	single, beg 96 hr post	sc	175-700	>700	-	>700	
1	Ribavirin	162	10-16-87	bid x 5, beg 24 hr post	po	0.32-150	>150	+	32	COMBINATION
1	Ribavirin	193	11-13-87	bid x 5, beg 24 hr post	po	0.32-150	>150	+	10	COMBINATION
1	Ribavirin	427	7-7-88	bid x 5, beg 24 hr post	po	1-200	>200	ON TEST	ON TEST	COMBINATION
1	Ribavirin	537	11/22/88	single, beg 24 hr post	ic	43.75-350	ON TEST	ON TEST	ON TEST	BALLIET
2	Ribavirin triacetate	106	8-14-87	bid x 5, beg 4 hr pre	sc	25-200	>200	+	25	
2	Ribavirin triacetate	112	8-21-87	bid x 5, beg 4 hr pre	sc	15.6-500	>500	TI 16	62.5	EXPANDED
2	Ribavirin triacetate	113	8-21-87	single, beg 4 hr post	sc	62.5-1000	>1000	+	62.5	
2	Ribavirin triacetate	114	8-21-87	single, beg 24 hr post	sc	62.5-1000	>1000	+	62.5	
2	Ribavirin triacetate	115	8-21-87	single, beg 48 hr post	sc	62.5-1000	>1000	+	62.5	
2	Ribavirin triacetate	116	8-21-87	single, beg 72 hr post	sc	62.5-1000	>1000	-	>1000	
2	Ribavirin triacetate	117	8-21-87	single, beg 96 hr post	sc	62.5-1000	>1000	-	>1000	
2	Ribavirin triacetate	134	9-18-87	bid x 5, beg 24 hr pre	po	9.4-600	600	TI 8	37.5	EXPANDED
2	Ribavirin triacetate	167	10-22-87	bid x 5, beg 4 hr pre	ip	125-1000	1000	+	250	BALLIET
2	Ribavirin triacetate	177	10-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>500	?		
2	Ribavirin triacetate	178	10-30-87	bid x 5, beg 4 hr pre	sc	62.5-500	>250	+	31.3	MMF
2	Ribavirin triacetate	179	10-30-87	bid x 5, beg 4 hr pre	sc	62.5-500	>250	+	62.5	MMF
2	Ribavirin triacetate	180	10-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>250	+	62.5	MMF
2	Ribavirin triacetate	181	10-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>250	+	62.5	MMF
2	Ribavirin triacetate	185	11-6-87	qd x 5, beg 4 hr pre	sc	31.3-1000	>1000	TI 16	62.5	
2	Ribavirin triacetate	339	4-15-88	single, beg 24 hr post	po	62.5-500	>500	+	62.5	EXPANDED
2	Ribavirin triacetate	340	4-15-88	single, beg 48 hr post	po	62.5-500	>500	+	250	EXPANDED
2	Ribavirin triacetate	377	5-20-88	bid x 5, beg 24 hr post	po	31.3-500	>500	+	31.3	EXPANDED
2	Ribavirin triacetate	378	5-20-88	bid x 5, beg 48 hr post	po	31.3-500	>500	+	31.3	EXPANDED
52	Thioformycin B	2	10-10-86	bid x 5, beg 4 hr pre	sc	62.5-250	>250	-	>250	

AVS #	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
52	Thioformycin B	22	1-22-87	single, beg 4 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	23	1-22-87	single, beg 8 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	24	1-22-87	single, beg 24 hr post	sc	300-1200	>1200	-	>1200	
52	Thioformycin B	153	10-9-87	tid x 5, beg 4 hr pre	sc	62.5-500	>500	+	250	
52	Thioformycin B	231A	12-18-87	qid x 5, beg 4 hr pre	sc	25-400	>400	±	50	
52	Thioformycin B	342	4-22-88	tid x 5, beg	po	50-400	>400	+	50	EXPANDED
65	Formycin B	52	3-12-87	bid x 5, beg 4 hr pre	sc	62.5-250	>250	-	>250	
65	Formycin B	551	12/1/88	tid x 5, beg 4 hr pre	sc	31.3-500	>500	+	125	
65	Formycin B	560	12/8/88	single, beg 4 hr pre	sc	31.3-500	>500	-	>500	
65	Formycin B	561	12/8/88	single, beg 24 hr post	sc	31.3-500	>500	+	62.5	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	3	10-10-86	bid x 5, beg 4 hr pre	sc	25-100	100	+	25	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	12	11-14-86	bid x 5, beg 4 hr pre	sc	6.25-50	>50	TI 2	6.25	EXPANDED
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	18	12-3-86	bid x 5, beg 24.4 hr pre	sc	9.4-75	>75	-	>75	BALLIET
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	25	1-22-87	single, beg 4 hr post	sc	175-700	700	?		
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	26	1-22-87	single, beg 8 hr post	sc	175-700	700	?		
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	27	1-22-87	single, beg 24 hr post	sc	175-700	700	?		
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	95	7-30-87	qd x 5, beg 4 hr pre	sc	25-200	200	?		
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	102	8-7-87	bid x 5, beg 4 hr pre	po	25-200	>200	±	>200	EXPANDED
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	107	8-14-87	bid x 5, beg 24 hr post	sc	18.8-150	>150	-	18.8	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	108	8-14-87	bid x 5, beg 36 hr post	sc	18.8-150	>150	-	37.5	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	109	8-14-87	bid x 5, beg 48 hr post	sc	18.8-150	>150	-	>150	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	133	9-11-87	qd x 5, beg 4 hr pre	sc	25-200	200	+	50	REPEAT #95
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	154	10-9-87	single, beg 4 hr post	sc	87.5-700	>700	±	350	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	155	10-9-87	single, beg 24 hr post	sc	87.5-700	>700	±	175	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	156	10-9-87	single, beg 48 hr post	sc	87.5-700	>700	+	87.5	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	157	10-9-87	single, beg 72 hr post	sc	87.5-700	>700	-	>700	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	158	10-9-87	single, beg 96 hr post	sc	87.5-700	>700	-	>700	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	187	11-6-87	bid x 5, beg 4 hr pre	ip	6.25-200	200	-	200	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	188	11-6-87	tid x 5, beg 4 hr pre	sc	6.25-200	200	+	6.25	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	336	4-15-88	single, beg 4 hr post	po	87.5-700	>700	±	175	EXPANDED
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	337	4-15-88	single, beg 24 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	338	4-15-88	single, beg 48 hr post	po	87.5-700	>700	+	87.5	EXPANDED
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	374	5/20/88	single, beg 4 hr post	po	87.5-700	>700	±	350	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	375	5/20/88	single, beg 24 hr post	po	87.5-700	>700	±	700	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	376	5/20/88	single, beg 48 hr post	po	87.5-700	>700	±	175	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	403	6-17-88	single, beg 60 hr post	po	43.8-700	>700	-	>700	
79	9-β-D-ribofuranosylpurine-6-thiocarboxamide	534	11/22/88	single, beg 24 hr post	ip	62.5-500	>500	-	>500	BALLIET
111	Tiazofurin	53	3-12-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	+	62.5	
111	Tiazofurin	68	3-26-87	bid x 5, beg 4 hr pre	sc	31.3-250	>500	+	31.3	EXPANDED
111	Tiazofurin	110	8-14-87	bid x 5, beg 4 hr pre	sc	15.7-2000	2000	TI=8-16	125	TI
111	Tiazofurin	135	9-18-87	single, beg 4 hr post	sc	125-1000	250	+	250	
111	Tiazofurin	136	9-18-87	single, beg 24 hr post	sc	125-1000	250	+	1000	
111	Tiazofurin	137	9-18-87	single, beg 48 hr post	sc	125-1000	250	+	250	
111	Tiazofurin	138	9-18-87	single, beg 72 hr post	sc	125-1000	250	-	>1000	
111	Tiazofurin	139	9-18-87	single, beg 96 hr post	sc	125-1000	250	±	1000	
111	Tiazofurin	182	11-5-87	bid x 5, beg 24 hr pre	sc	62.5-500	>500	-	>500	BALLIET
111	Tiazofurin	365	5-6-88	bid x 5, beg 4 hr pre	po	93.8-750	>750	+	93.8	EXPANDED
147	Enviroxime	15	11-19-86	bid x 5, beg 4 hr pre	sc	25-100	>100	-	>100	

AVS #	Compound Name	Expt #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
147	Enviroxime	34	1-29-87	single, beg 4 hr post	sc	250-1000	>1000	+	1000	
147	Enviroxime	35	1-29-87	single, beg 12 hr post	sc	250-1000	>1000	±	>1000	
147	Enviroxime	36	1-29-87	single, beg 24 hr post	sc	250-1000	>1000	-	>1000	
147	Enviroxime	96	7-30-87	qd x 5, beg 4 hr pre	sc	62.5-500	>500	-	>500	
147	Enviroxime	371	5-13-88	single, beg 4 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	372	5-13-88	single, beg 24 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	373	5-13-88	single, beg 48 hr post	po	125-1000	>1000	-	BAD TEST	EXPANDED
147	Enviroxime	522	11/2/88	single, beg 24 hr pre	po	150-1200	1200	-	>1200	EXPANDED
147	Enviroxime	523	11/3/88	single, beg 4 hr post	po	150-1200	1200	±	300	EXPANDED
147	Enviroxime	524	11/3/88	single, beg 24 hr post	po	150-1200	1200	-	>1200	EXPANDED
167	Glycerrhetic Acid	54	3-12-87	bid x 5, beg 4 hr pre	sc	18.8-75	>75	-	>75	
167	Glycerrhetic Acid	87	4-24-87	bid x 5, beg 4 hr pre	sc	62.5-500	>500	-	500	REPEAT
167	Glycerrhetic Acid	304	3-3-88	tid x 5, beg 24 hr pre	ip	75-600	300	-	>600	
206	Ribamidine	4	10-10-86	bid x 5, beg 4 hr pre	sc	125-500	>500	+	125	
206	Ribamidine	13	11-14-86	bid x 5, beg 4 hr pre	sc	31.3-250	>250	+	31.3	
206	Ribamidine	71	4-3-87	bid x 5, beg 4 hr pre	sc	3.9-1000	>1000	TI >32	31.3	TI
206	Ribamidine	78	4-10-87	bid x 5, beg 24 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	79	4-10-87	bid x 5, beg 36 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	80	4-10-87	bid x 5, beg 48 hr post	sc	62.5-500	>500	+	62.5	EXPANDED
206	Ribamidine	81	4-10-87	bid x 5, beg 72 hr post	sc	62.5-500	>500	+	125	EXPANDED
206	Ribamidine	86	4-23-87	bid x 5, beg 24 hr pre	sc	125-500	>500	-	125	BALLIET
206	Ribamidine	92	7-28-87	bid x 5, beg 4 hr pre	po	7.8-1000	>1000	TI =64	31.3	TI
206	Ribamidine	169	10-23-87	single, beg 4 hr post	sc	15.7-1000	>1000	+	62.5	
206	Ribamidine	170	10-23-87	single, beg 24 hr post	sc	15.7-1000	>1000	+	500	
206	Ribamidine	171	10-23-87	single, beg 48 hr post	sc	15.7-1000	>1000	+	250	
206	Ribamidine	172	10-23-87	single, beg 72 hr post	sc	15.7-1000	>1000	-	>1000	
206	Ribamidine	173	10-23-87	single, beg 96 hr post	sc	15.7-1000	>1000	-	>1000	
206	Ribamidine	233	12-18-87	bid x 5, beg 4 hr pre	sc	7.8-2000	2000	+		
206	Ribamidine	234	12-18-87	bid x 5, beg 4 hr pre	po	7.8-2000	2000	+		
206	Ribamidine	287	2-19-88	bid x 5, beg 24 hr post	po	2.4-75	>75	+	2.4	COMBINATION
206	Ribamidine	363	5-6-88	bid x 5, beg 24 hr pre	ip	75-600	>600	±	600	BALLIET
206	Ribamidine	382	5-27-88	bid x 5, beg 18 hr post	po	2.4-75	>75	+	18.8	COMBINATION
206	Ribamidine	447	8-5-88	bid x 3, beg 24 hr post	po	1000	>1000	ON TEST	ON TEST	
206	Ribamidine	535	11/22/88	single, beg 24 hr post	ip	250-2000	>2000	±	2000	BALLIET
206	Ribamidine	536	11/22/88	single, beg 24 hr post	ic	62.5-1000	500	-	>1000	BALLIET
212	Suramin	18	11-19-86	bid x 5, beg 4 hr pre	sc	18.8-75	>25	-	>75	
212	Suramin	37	1-29-87	single, beg 4 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	38	1-29-87	single, beg 12 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	39	1-29-87	single, beg 24 hr post	sc	250-1000	>600	-	>1000	
212	Suramin	103	8-7-87	bid x 5, beg 4 hr pre	po	75-200	>200	-	>200	EXPANDED
212	Suramin	159	10-9-87	tid x 5, beg 4 hr pre	sc	18.8-150	>150	-	>150	
215	3-Deazaguanosine	497	10-13-88	qd x 5, beg 4 hr pre	sc	18.8-300	150	±	37.5	
215	3-Deazaguanosine	557	12/8/88	tid x 5, beg 4 hr pre	sc	12.5-100	>100	-	>100	
215	3-Deazaguanosine	558	12/8/88	bid x 5, beg 4 hr pre	sc	12.5-100	>100	+	50	
215	3-Deazaguanosine	559	12/8/88	bid x 5, beg 4 hr pre	ip	12.5-100	>100	+	25	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	55	3-12-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	-	>250	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	88	4-24-87	bid x 5, beg 4 hr pre	sc	31.3-250	>250	-	31.3	EXPANDED
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	302	3-3-88	qd x 5, beg 24 hr pre	sc	62.5-500	>500	-	>500	

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222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	366	5-6-88	bid x 5, beg 4 hr pre	sc	2000	2000	-	>2000	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	437	7-20-88	single, 24 hr pre	ip	187.5-1500	>1500	±	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	438	7-21-88	single, 4 hr pre	ip	187.5-1500	>1500	-	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	439	7-21-88	single, 24 hr post	ip	187.5-1500	>1500	-	>1500	
222	3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine	440	7-21-88	tid x 5, beg 4 hr pre	sc	62.5-500	>500	-	>500	
233	Formycin	17	11-19-86	bid x 5, beg 4 hr pre	sc	100-400	>400	-	>400	
233	Formycin	40	1-29-87	single, beg 12 hr post	sc	450-1800	900	±	450	
233	Formycin	41	1-29-87	single, beg 12 hr post	sc	450-1800	900	±	450	
253	Selenazofurin	5	10-10-86	bid x 5, beg 4 hr pre	sc	80-320	160	+	80	
253	Selenazofurin	14	11-14-86	bid x 5, beg 4 hr pre	sc	20-160	>160	+	80	
253	Selenazofurin	19	12-3-86	bid x 5, beg 24,4 hr pre	sc	18.8-150	>150	+	>150	BALLIET
253	Selenazofurin	97	7-30-87	qd x 5, beg 4 hr pre	sc	40-320	320	±	40	REPEAT
253	Selenazofurin	104	8-7-87	bid x 5, beg 4 hr pre	po	40-320	320	+	40	EXPANDED
257	Tiazofurin 5'-MP	445	7-21-88	bid x 5, beg 4 hr pre	ip	93.75-750	>750	±	93.8	BALLIET
257	Tiazofurin 5'-MP	449	9-2-88	bid x 5, beg 4 hr pre	ip	25-400	>400	+	400	
272	3-Deazaguanine	186	11-6-87	bid x 5, beg 4 hr pre	ip	50-400	>400	+	200	EXPANDED
272	3-Deazaguanine	232	12-18-87	qd x 5, beg 4 hr pre	sc	25-200	100	-	>200	
272	3-Deazaguanine	260	2-11-88	bid x 5, beg 24 hr pre	sc	25-200	>200	+	25	
272	3-Deazaguanine	317	3-18-88	bid x 5, beg 24 hr pre	ip	12.5-100	>100	?		
272	3-Deazaguanine	343	4-22-88	qd x 5, beg	ip	12.5-100	100	-	>12.5	
272	3-Deazaguanine	370	5-13-88	qd x 5, beg 4 hr pre	ip	25-200	200	-	>200	BALLIET
272	3-Deazaguanine	498	10-13-88	qd x 5, beg 4 hr pre	po	18.8-300	>300	+	18.8	EXPANDED
272	3-Deazaguanine	539	11/22/88	single, beg 4 hr post	sc	18.8-300	>300	-	>300	EXPANDED
360	7-Deoxynarciclasin	42	1-29-87	bid x 5, beg 4 hr pre	ip	93.75-750	>750	-	>750	BALLIET
361	Pancratistatin	417	6-24-88	qd x 7, beg 24 hr pre	sc	62.5-500	>500	±	7.5	
1212	Uridine 2',3'-dialdehyde	550	12/1/88	bid x 5, beg 4 hr pre	sc	.5-4	>4	-	>4	
1212	Uridine 2',3'-dialdehyde	562	12/8/88	single, beg 4 hr pre	sc	25-400	>400	-	>400	INITIAL
1212	Uridine 2',3'-dialdehyde	563	12/8/88	single, beg 24 hr post	sc	25-400	>400	±	25	
1754	MVE-2	58	3-19-87	single, beg 24 hr pre	ip	25-400	>400	+	25	
1754	MVE-2	89	4-23-87	single, beg 24 hr pre	ip	6.25-50	>50	+	12.5	
1754	MVE-2	98	7-30-87	single, beg 4 hr pre	ip	6.25-50	>50	+	6.25	EXPANDED
1754	MVE-2	99	7-30-87	single, beg 4 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	100	7-30-87	single, beg 24 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	101	7-30-87	single, beg 48 hr post	ip	6.25-100	25	+	6.3	
1754	MVE-2	151	10-1-87	single, beg 24 hr pre	po	6.25-100	25	+	6.3	
1754	MVE-2	161	10-6-87	single, beg 4 hr pre	ip	6.25-200	>200	-	>200	EXPANDED
1754	MVE-2	238	1/8/88	qd x 3, beg 4 hr pre	ip	12.5-100	100	±	50	BALLIET
1754	MVE-2	240	1/8/88	single, beg 72 hr post	ip	3.13-50	50	+	6.25	
1754	MVE-2	241	1/8/88	single, beg 96 hr post	ip	6.25-100	>100	-	>100	
1754	MVE-2	249	1/15/88	single, beg 4 hr pre	ip	6.25-100	>100	-	>100	
1754	MVE-2	252	1-14-88	single	sc	6.25-100	12.5	+	6.25	
1754	MVE-2	311	3-11-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	12.5	IFN
1754	MVE-2	431	7-7-88	single, beg 24 hr post	ip	6.25-50	>50	+	6.25	
1757	Isoprinosine	76	4-10-87	bid x 5, beg 24 hr post	ip	0.05, 0.5, 5	>5	+	5	IFN, EXPANDED
1761	Poly IC•LC	307	3-3-88	qd x 8, beg 24 hr pre	po	250-1000	>1000	-	>1000	
1761	Poly IC•LC	324	3-24-88	qd x 8, beg 24 hr pre	ip	0.0195-5	5	+	0.0195	
1761	Poly IC•LC	325	3-24-88	qd x 8, beg 24 hr pre	sc	0.031-1	>1	+	0.031	EXPANDED
					po	0.031-1	>1	-	1	EXPANDED

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1761	Poly IC·LC	326	3-25-88	single, beg 4 hr pre	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC·LC	327	3-25-88	single, beg 4 hr post	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC·LC	328	3-25-88	single, beg 24 hr post	ip	0.31-2.5	>2.5	+	0.31	
1761	Poly IC·LC	329	3-25-88	single, beg 48 hr post	ip	0.31-2.5	>2.5	+	0.625	
1761	Poly IC·LC	330	3-25-88	single, beg 72 hr post	ip	0.31-2.5	>2.5	-	>2.5	
1761	Poly IC·LC	331	3-25-88	single, beg 96 hr post	ip	0.31-2.5	>2.5	-	>2.5	
1761	Poly IC·LC	351	4-29-88	qd x 5, beg 4 hr pre	sc	0.0625-0.5	>0.5	-	>0.5	BALLIET
1767	AM-3	72	4-3-87	bid x 5, beg 4 hr pre	sc	112.5 450	>450	+	112.5	
1767	AM-3	73	4-3-87	bid x 5, beg 4 hr pre	po	112.5-450	>450	-	>450	
1767	AM-3	111	8-14-87	bid x 5, beg 4 hr pre	sc	62.5-2000	2000	+	62.5	EXPANDED
1767	AM-3	168	10-22-87	bid x 5, beg 24 hr pre	ip	62.5-500	500	-	>500	BALLIET
1767	AM-3	243	1/15/88	single, beg 4 hr pre	sc	25-400	>400	+	50	
1767	AM-3	244	1/15/88	single, beg 4 hr post	sc	25-400	>400	+	25	
1767	AM-3	245	1/15/88	single, beg 24 hr post	sc	25-400	>400	+	25	
1767	AM-3	246	1/15/88	single, beg 48 hr post	sc	25-400	>400	+	25	
1767	AM-3	247	1/15/88	single, beg 72 hr post	sc	25-400	>400	-	>400	
1767	AM-3	248	1/15/88	single, beg 96 hr post	sc	25-400	>400	-	>400	
1767	AM-3	251	1-14-88	single	sc	25-400	>400	-	>400	IFN
1767	AM-3	259	1-29-88	qd x 5, beg 4 hr pre	sc	31.3-250	>250	+	62.5	
1767	AM-3	260	1-29-88	single, beg 4 hr pre	sc	15.6-1000	1000	+	15.6	
1767	AM-3	261	1-29-88	single, beg 4 hr post	sc	15.6-1000	1000	+	62.5	
1767	AM-3	262	1-29-88	single, beg 24 hr post	sc	15.6-1000	1000	±	62.5	
1767	AM-3	263	1-29-88	single, beg 48 hr post	sc	15.6-1000	1000	+	15.6	
1767	AM-3	264	1-29-88	single, beg 72 hr post	sc	15.6-1000	1000	±	500	
1767	AM-3	265	1-29-88	single, beg 96 hr post	sc	15.6-1000	1000	±	15.6	
1767	AM-3	267	1-29-88	single	sc	31.3-250	>250	-	>250	IFN
1767	AM-3	308	3-11-88	bid x 5, beg 4 hr pre	ip	15.7-250	>250	+	15.7	
1767	AM-3	386	5-27-88	single, beg 48 hr post	sc	5, 16, 50	>50	-	50	COMBINATION
1767	AM-3	540	11/22/88	single, beg 4 hr post	sc	62.5-500	>500	-	>500	BALLIET
1777	Streptonigrin	77	4-10-87	qd x 5, beg 4 hr pre	sc	0.125-1	0.5	-	>1	
1778	Mannozym	74	4-3-87	single, beg 4 hr pre	sc	12.5-50	50	+	25	
1778	Mannozym	75	4-3-87	bid x 5, beg 4 hr pre	sc	3.1-50	>50	+	3.13	
1778	Mannozym	93	7-28-87	bid x 5, beg 4 hr pre	po	9.4-150	>150	-	>150	
1778	Mannozym	118	8-28-87	bid x 5, beg 4 hr pre	sc	1.6-100	>100	+	3.1	EXPANDED
1778	Mannozym	119	8-28-87	bid x 5, beg 4 hr pre	po	1.6-100	>100	-	>100	EXPANDED
1778	Mannozym	152	10-2-87	bid x 5, beg 4 hr pre	sc	6.25-100	>100	-	>100	BALLIET
1778	Mannozym	198	11-19-87	single, beg 24 hr pre	sc	6.3-50	>50	-	>50	
1778	Mannozym	199	11-19-87	single, beg 4 hr pre	sc	6.3-50	>50	-	>50	
1778	Mannozym	200	11-19-87	single, beg 4 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	201	11-19-87	single, beg 24 hr post	sc	6.3-50	>50	-	25	
1778	Mannozym	202	11-19-87	single, beg 48 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	203	11-19-87	single, beg 72 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	204	11-19-87	single, beg 96 hr post	sc	6.3-50	>50	-	>50	
1778	Mannozym	216	12-4-87	qd x 5, beg 4 hr pre	sc	3.13-100	>100	±	3.13	
1778	Mannozym	217	12-4-87	bid x 5, beg 4 hr pre	ip	0.78-400	200	-	>100	
1778	Mannozym	239	1/8/88	qd x 5, beg 4 hr pre	sc	9.4-150	>150	?		
1778	Mannozym	250	1/15/88	single, beg 4 hr pre	sc	6.25-100	12.5	±	50	
1778	Mannozym	253	1-14-88	single	sc	6.75-100	>100	-	>100	IFN

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1778	Mannozym	293	2-26-88	qd x 5, beg 4 hr pre	sc	9.4-150	>150	+	9.4	
1778	Mannozym	294	2-26-88	bid x 5, beg 4 hr pre	sc	1.6-50	>50	+	1.6	
1778	Mannozym	295	2-26-88	bid x 5, beg 24 hr post	sc	9.4-150	>150	+	1.6	
1778	Mannozym	296	2-26-88	bid x 5, beg 48 hr post	sc	9.4-150	>150	+	3.2	
1969	CL259763	356	4-29-88	single, beg 24 hr pre	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	357	4-29-88	single, beg 4 hr pre	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	358	4-29-88	single, beg 24 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	359	4-29-88	single, beg 48 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	360	4-29-88	single, beg 72 hr post	po	2, 20, 200	>200	±	2	EXPANDED
1969	CL259763	391	6-9-88	single, beg 24 hr pre	ip	2, 20, 200	>200	±	20	
1969	CL259763	392	6-9-88	single, beg 4 hr pre	ip	2, 20, 200	>200	-	>200	
1969	CL259763	393	6-9-88	single, beg 48 hr post	ip	2, 20, 200	>200	-	>200	
1969	CL259763	394	6-9-88	single, beg 24 hr post	ip	2, 20, 200	>200	-	>200	
1969	CL259763	395	6-9-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	25	
1969	CL259763	425	7-1-88	bid x 5, beg 4 hr pre	po	2, 20, 200	>200	-	>200	
1969	CL259763	434	7-13-88	single, beg 24 hr pre	ip	5-80	>80	±	5	EXPANDED
1969	CL259763	436	7-13-88	eod x 3, beg 24 hr pre	ip	2-200	>200	-	>200	IFN
1969	CL259763	541	11/22/88	single, beg 4 hr post	ip	50-400	>400	-	>400	BALLIET
1976	Thymine riboside 2',3'-dialdehyde	446	7-21-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	-	>100	
1976	Thymine riboside 2',3'-dialdehyde	452	9-2-88	single, beg 24 hr pre	ip	62.5-500	500	±	62.5	
1976	Thymine riboside 2',3'-dialdehyde	453	9-2-88	single, beg 4 hr pre	ip	62.5-500	500	±	62.5	
1976	Thymine riboside 2',3'-dialdehyde	454	9-2-88	single, beg 24 hr post	ip	62.5-500	500	-	>500	
1976	Thymine riboside 2',3'-dialdehyde	481	9-30-88	bid x 5, beg 4 hr pre	ip	50-400	400	-	>400	
2149	Ampligen	56	3-12-87	qd x 8, beg 24 hr pre	ip	0.6-5	>5	+	0.625	
2149	Ampligen	57	3-12-87	eod x 8, beg 24 hr pre	sc	0.6-5	>5	+	0.625	
2149	Ampligen	69	3-26-87	qd x 8, beg 24 hr pre	sc	0.6-5	>5	+	0.313	EXPANDED
2149	Ampligen	128	9-10-87	qd x 5, beg 24 hr pre	ip	0.6-5	>5	+	0.625	
2149	Ampligen	129	9-10-87	qd x 5, beg 4 hr pre	ip	0.6-5	>5	+	0.625	
2149	Ampligen	130	9-10-87	qd x 5, beg 4 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	131	9-10-87	qd x 5, beg 24 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	132	9-10-87	qd x 5, beg 48 hr post	ip	0.6-5	>5	+	0.625	
2149	Ampligen	142	9-25-87	qd x 5, beg 4 hr pre	po	0.04-5	>5	±	0.039	EXPANDED
2149	Ampligen	160	10-8-87	qd x 5, beg 4 hr pre	ip	0.625-5	>5	+	0.63	BALLIET
2149	Ampligen	166	10-16-87	qd x 5, beg 24 hr post	ip	0.05-5	>5	+	0.05	COMBINATION
2149	Ampligen	195	11-13-87	qd x 5, beg 24 hr post	ip	0.005	>0.005	±	0.005	COMBINATION
2149	Ampligen	205	11-20-87	bid x 5, beg 4 hr pre	ip	0.31-5	>5	+	0.625	
2149	Ampligen	207	12-4-87	qd x 5, beg 4 hr pre	ip	3.13-25	>25	+	3.13	TI
2149	Ampligen	208	12-4-87	single, beg 4 hr pre	ip	1.25-10	>10	+	1.25	
2149	Ampligen	209	12-3-87	single, beg 24 hr pre	ip	1.25-10	>10	+	1.25	
2149	Ampligen	210	12-4-87	single, beg 4 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	211	12-4-87	single, beg 24 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	212	12-4-87	single, beg 48 hr post	ip	1.25-10	>10	+	1.25	
2149	Ampligen	213	12-4-87	single, beg 72 hr post	ip	1.25-10	>10	-	>10	
2149	Ampligen	214	12-4-87	single, beg 96 hr post	ip	1.25-10	>10	-	>10	
2149	Ampligen	215	12-3-87	bid x 5, beg 24 hr pre	ip	0.6-5	>5	+	0.6	
2149	Ampligen	242	1-7-88	single	ip	0.05, 0.5, 5	>5	-		IFN
2149	Ampligen	257	1/22/88	qd x 5, beg 4 hr pre	ip	0.31-5	>5	+	0.31	
2149	Ampligen	309	3-11-88	qd x 5, beg 72 hr post	ip	0.625-5	>5	-	>5	

AVS #	Compound Name	Expt. #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
2149	Ampligen	310	3-11-88	qd x 5, beg 96 hr post	ip	0.625-5	>5	-	>5	
2149	Ampligen	362	5-6-88	bid x 5, beg 4 hr pre	ip	0.625-5	>5	-	>5	BALLIET
2149	Ampligen	407	6-17-88	qd x 5, beg 4 hr pre	ip	0.6-5	>5	ON TEST	ON TEST	IFN
2149	Ampligen	408	6-17-88	single, beg 48 hr post	ip	0.6-5	>5	ON TEST	ON TEST	IFN
2149	Ampligen	409	6-17-88	bid x 5, beg 4 hr pre	ip	0.6-5	>5	ON TEST	ON TEST	IFN
2700	6-Ethyl thiopurine riboside	432	7-14-88	bid x 5, beg 4 hr pre	ip	25-400	400	+	25	
2700	6-Ethyl thiopurine riboside	450	9-2-88	bid x 5, beg 4 hr pre	ip	3.13-100	>100	±	~50	EXPANDED
2700	6-Ethyl thiopurine riboside	473	9-22-88	bid x 5, beg 4 hr pre	ip	1.56-50	>50	±	~50	EXPANDED
2712	Bryostatin 1	305	3-4-88	qd x 5, beg 4 hr pre	ip	4.5-36	>36	±	18	
2712	Bryostatin 1	379	5/20/88	bid x 5, beg 4 hr pre	ip	6.25-50	>50	±	12.5	
2712	Bryostatin 1	426	7-1-88	qd x 5, beg 4 hr pre	ip	2.25-18 µg/ml	>18	-	>18	
2712	Bryostatin 1	503	10/20/88	qd x 5, beg 4 hr pre	ip	4.5-144 µg/ml	>144	-	>144	
2712	Bryostatin 1	509	10/26/88	single, beg 24 hr pre	ip	6.25-200 µg/ml	>200	+	12.5	
2712	Bryostatin 1	510	10/27/88	single, beg 4 hr post	ip	6.25-200 µg/ml	>200	+	12.5	
2712	Bryostatin 1	556	12/8/88	tid x 5, beg 4 hr pre	ip	1.13-18 µg/ml	>18	+	2.3	
2713	Bryostatin 2	306	3-4-88	qd x 5, beg 4 hr pre	ip	4.5-36	>36	-	>36	
2713	Bryostatin 2	380	5/20/88	bid x 5, beg 4 hr pre	ip	5-40	>40	-	>40	
2741	Ribavirin tetrahydropyrimidine	149	10-2-87	bid x 5, beg 4 hr pre	sc	31.3-500	>500	-	>500	
2741	Ribavirin tetrahydropyrimidine	297	2-26-88	bid x 5, beg 4 hr pre	sc	75-600	>600	-	>600	
2742	Ribavirin 5-OH tetrahydropyrimidine	150	10-2-87	bid x 5, beg 4 hr pre	sc	31.3-500	>500	±	500	
2776	Bropirimine	59	3-19-87	qd x 3, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	60	3-19-87	single, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	61	3-19-87	e 3 days x 3, beg 24 hr pre	ip	50-400	400	+	100	
2776	Bropirimine	90	4-23-87	single, beg 24 hr pre	ip	100-400	400	+	100	EXPANDED
2776	Bropirimine	143	9-25-87	single, beg 4 hr pre	ip	100-400	>400	+	100	
2776	Bropirimine	144	9-25-87	single, beg 4 hr post	ip	100-400	>400	+	200	
2776	Bropirimine	145	9-25-87	single, beg 24 hr post	ip	100-400	>400	+	200	
2776	Bropirimine	146	9-25-87	single, beg 48 hr post	ip	100-400	>400	+	400	
2776	Bropirimine	147	9-25-87	single, beg 72 hr post	ip	100-400	>400	-	>400	
2776	Bropirimine	148	9-25-87	single, beg 96 hr post	ip	100-400	>400	-	400	
2776	Bropirimine	254	1-21-88	qd x 3, beg 24 hr pre	po	25-400	400	+	25	EXPANDED
2776	Bropirimine	255	1-21-88	single, beg 24 hr pre	po	25-400	>400	+	25	EXPANDED
2776	Bropirimine	256	1/21/88	single, beg 24 hr pre	sc	50-400	200	+	200	
2776	Bropirimine	291	2-19-88	single, beg 24 hr post	po	25-100	>100	+	50	COMBINATION
2776	Bropirimine	312	3-11-88	qd x 3, beg 24 hr post	po	12.5-200	>200	+	12.5	EXPANDED
2776	Bropirimine	364	5-6-88	single, beg 4 hr pre	ip	50-400	>400	+	100	BALLIET
2776	Bropirimine	413	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	414	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	415	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	416	6-24-88	single, beg 24 hr post	po	25-400	>400	+	50	MMF
2776	Bropirimine	448	8-5-88	single, beg 24 hr post	po	400	>400	+	400	IFN
2776	Bropirimine	474	9-22-88	single, beg 24 hr post	po	25-400	>400	ON TEST	ON TEST	MMF
2776	Bropirimine	475	9-22-88	single, beg 24 hr post	po	25-400	>400	ON TEST	ON TEST	MMF
2776	Bropirimine	476	9-22-88	single, beg 24 hr post	po	25-400	>400	ON TEST	ON TEST	MMF
2776	Bropirimine	477	9-22-88	single, beg 24 hr post	po	25-400	>400	ON TEST	ON TEST	MMF
2776	Bropirimine	549	11/30/88	single, beg 48 hr post	ip	200	ON TEST	ON TEST	ON TEST	IMMUNOLOGY
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	62	3-19-87	qd x 3, beg 24 hr pre	ip	50-400	400	+	200	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	63	3-19-87	single, beg 24 hr pre	ip	50-400	400	+	>400	

AVS #	Compound Name	Expt. #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	64	3-19-87	e 3 days x 3, beg 24 hr pre	ip	50-400	>400	+	400	
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	91	5-23-87	single, beg 24 hr pre	ip	100-400	200	±	100	EXPANDED
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	174	10-29-87	qd x 3, beg 24 hr pre	ip	37.5-300	>300	-	>300	BALLIET
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	231	12-10-87	qd x 3, beg 24 hr pre	po	50-400	200	+	50	EXPANDED
2777	2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone (AIPP)	313	3/11/88	single, beg 4 hr pre	po	25-200	>200	±	25	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP)	65	3-26-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP)	66	3-26-87	single, beg 24 hr pre	ip	50-400	>400	+	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP)	67	3-26-87	e 3 days x 3, beg 24 hr pre	ip	50-400	>400	+	50	
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP)	235	1-7-88	single, beg 24 hr pre	ip	50-800	>800	+	50	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP)	274	2-4-88	single, beg 24 hr pre	po	50-400	200	+	50	EXPANDED
2778	2-Amino-5-bromo-methyl-4(3H)-pyrimidinone (ABMP)	333	3-31-88	qd x 3, beg 24 hr pre	po	12.5-400	200	+	12.5	EXPANDED
2779	MVE-1	500	10/19/88	single, beg 24 hr pre	ip	6.25-100	>100	+	6.25	
2779	MVE-1	501	10/20/88	single, beg 4 hr pre	ip	6.25-100	>100	+	6.25	
2779	MVE-1	502	10/20/88	single, beg 24 hr post	ip	6.25-100	>100	+	12.5	
2779	MVE-1	543	11/22/88	single, beg 24 hr post	ip	12.5-100	>100	-	>100	BALLIET
2811	7-Deoxynarciclasine	554	12/1/88	single, beg 4 hr pre	ip	0.78-100	ON TEST	ON TEST	ON TEST	EXPANDED
2811	7-Deoxynarciclasine	236	1/8/98	qd x 5, beg 4 hr pre	ip	3.13-25	>25	-	>25	
2812	Narciclasine	369	5/13/88	bid x 5, beg 4 hr pre	ip	1-8	>8	±	4	
2812	Narciclasine	237	1/8/88	qd x 5, beg 4 hr pre	ip	0.75-6	>6	+	6	
2880	Oxamisole	292	2-26-88	qd x 5, beg 4 hr pre	ip	0.75-12	>12	+	0.75	EXPANDED
2880	Oxamisole	82	4-16-87	qd x 3, beg 24 hr pre	ip	1.6-25	>25	±	1.6	
2880	Oxamisole	83	4-16-87	qd x 3, beg 24 hr post	ip	1.6-25	>25	-	>25	
2880	Oxamisole	84	4-17-87	single, beg 24 hr post	ip	1.6-50	>50	±	25	
2880	Oxamisole	105	8-6-87	bid x 3, beg 24 hr pre	po	1.6-25	>25	±	1.56	EXPANDED
2880	Oxamisole	183	11-5-87	qd x 3, beg 24 hr pre	ip	1.55-25	>25	±	1.55	BALLIET
2880	Oxamisole	184	11-5-87	single, beg 24 hr pre	ip	3.13-50	50	±	25	BALLIET
2880	Oxamisole	206	11-19-87	bid x 3, beg 24 hr pre	ip	0.78-25	>25	±	1.56	
2880	Oxamisole	258	1/21/88	qd x 2, beg 24 hr pre	ip	0.78-50	50	±	0.78	
2880	Oxamisole	268	2-5-88	qd x 2, beg 4 hr pre	ip	0.78-50	>50	-	>50	
2880	Oxamisole	269	2-5-88	qd x 2, beg 4 hr post	ip	0.78-50	>50	±	25	
2880	Oxamisole	270	2-5-88	qd x 2, beg 24 hr post	ip	0.78-50	>50	-	>50	
2880	Oxamisole	271	2-5-88	qd x 2, beg 48 hr post	ip	0.78-50	>50	±	1.56	
2880	Oxamisole	272	2-4-88	e 3 day x 3, beg 24 hr pre	ip	0.78-50	>50	-	>50	
2880	Oxamisole	273	2-4-88	single	ip	3.13-50	>50	-	>50	IFN
2880	Oxamisole	334	4-1-88	qd x 3, beg 4 hr post	po	0.76-50	>50	±	0.76	
2880	Oxamisole	335	4-6-88	qd x 3, beg 24 hr pre	ip	1.5-25	>25	-	>25	
2933	CGP 19835 A Lipid	350	4-29-88	single, beg 48 hr pre	ip	10,100,1000µ	>1000 µg	±	1000	
2933	CGP 19835 A Lipid	351	4-29-88	single, beg 24 hr pre	ip	10,100,1000µ	>1000 µg	±	10	
2933	CGP 19835 A Lipid	352	4-29-88	single, beg 4 hr pre	ip	10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	353	4-29-88	single, beg 24 hr post	ip	10,100,1000µ	>1000 µg	+	100	
2933	CGP 19835 A Lipid	354	4-29-88	single, beg 48 hr post	ip	10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	355	4-29-88	single, beg 72 hr post	ip	10,100,1000µ	>1000 µg	±	1000	
2933	CGP 19835 A Lipid	402	6-9-88	eod x 3, beg 24 hr pre	ip	1,10,100,1000µ	>1000 µg	-	>1000	
2933	CGP 19835 A Lipid	410	6-17-88	single, beg 24 hr post	ip	313-10000 µ	>10,000	+	313	EXPANDED
2933	CGP 19835 A Lipid	455	9-9-88	single, beg 24 hr post	sc	600-4800 µ	>4800 µg	-	>4800	BALLIET
2978	Tetraacetate ester of 2980	298	2-26-88	bid x 5, beg 4 hr pre	sc	25-200	>200	±	50	
2978	Tetraacetate ester of 2980	332	4-1-88	bid x 5, 4 hr pre	ip	25-400	>400	-	>400	
2980	Tetrahydroxy analog of Pancratistatin	266	1-29-88	bid x 5, beg 4 hr pre	sc	31.3-500	31.3	-	>500	

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2980	Tetrahydroxy analog of Pancratistatin	396	6-10-88	single, beg 4 hr pre	ip	6.25-50	>50	-	>50	
2980	Tetrahydroxy analog of Pancratistatin	397	6-10-88	single, beg 4 hr post	ip	6.25-50	>50	-	>50	
2980	Tetrahydroxy analog of Pancratistatin	398	6-10-88	single, beg 24 hr post	ip	6.25-50	>50	-	>50	
3425	8-Bromoguanosine	451	9-2-88	bid x 5, beg 4 hr pre	ip	15.6-500	500	-	>500	
3425	8-Bromoguanosine	491	10-12-88	single, beg 24 hr pre	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	492	10-12-88	single, beg 4 hr post	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	493	10-12-88	single, beg 24 hr post	sc	15.6-250	~250	-	>250	
3425	8-Bromoguanosine	505	10/27/88	qd x 5, beg 4 hr pre	sc	25-200	>200	±	50	
3425	8-Bromoguanosine	506	10/26/88	single, beg 24 hr pre	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	507	10/27/88	single, beg 4 hr post	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	508	10/27/88	single, beg 24 hr post	sc	50-400	400	-	>400	
3425	8-Bromoguanosine	525	11/2/88	bid x 5, beg 4 hr pre	po	15.6-250	>250	-	>250	
3425	8-Bromoguanosine	526	11/9/88	single, beg 24 hr pre	sc	100-800	800	+	100	
3425	8-Bromoguanosine	527	11/10/88	single, beg 4 hr post	sc	100-800	800	+	100	
3425	8-Bromoguanosine	528	11/10/88	single, beg 24 hr post	sc	100-800	800	±	800	
3425	8-Bromoguanosine	564	12/8/88	qd x 5, beg 4 hr pre	sc	15.7-250	>250	-	>250	
3580	UNIDENTIFIED	404	6-17-88	bid x 5, beg 4 hr pre	ip	6.25-100	>100	±	25	
3580	UNIDENTIFIED	532	11/9/88	single, beg 24 hr pre	sc	37.5-300	>300	±	37.5	
3580	UNIDENTIFIED	533	11/10/88	single, beg 4 hr post	sc	37.5-300	>300	-	>300	
3585	Neurotropin	126	9-3-87	qd x 3 days sep., beg 24 pre	ip	3-24	>24	-	>400	
3585	Neurotropin	127	9-3-87	single, beg 24 hr pre	ip	3-24	>24	-	>400	
3585	Neurotropin	140	9-24-87	qd x 3, beg 24 hr pre	ip	3-24	>24	-	>24	
3585	Neurotropin	141	9-24-87	eod x 3, beg 24 hr pre	ip	3-24	>24	-	>24	
3585	Neurotropin	278	2-11-88	single, beg 24 hr pre	po	3-24		?		
3585	Neurotropin	316	3/17/88	single, beg 24 hr pre	po	3-24	>24	-	>24	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	120	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	400	-	>400	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	121	9-3-87	single, beg 24 hr pre	ip	50-400	400	+	100	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	399	6-10-88	single, beg 4 hr pre	ip	50-400	>400	-	>400	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	400	6-10-88	single, beg 4 hr post	ip	50-400	>400	+	100	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	401	6-10-88	single, beg 24 hr post	ip	50-400	>400	+	50	
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	435	7-14-88	single, beg 4 hr post	ip	31.3-500	>500	±	31.3	EXPANDED
3587	2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone	457	9-8-88	single, beg 4 hr post	po	31.3-500	500	±	125	EXPANDED
3588	Meta Fluoro ABPP	122	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	200	+	100	
3588	Meta Fluoro ABPP	123	9-3-87	single, beg 24 hr pre	ip	50-400	100	+	100	
3588	Meta Fluoro ABPP	175	10-29-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	50	BALLIET
3588	Meta Fluoro ABPP	281	2-12-88	single, beg 4 hr pre	ip	50-400	400	?		
3588	Meta Fluoro ABPP	282	2-12-88	single, beg 4 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	283	2-12-88	single, beg 24 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	284	2-12-88	single, beg 48 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	285	2-12-88	single, beg 72 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	286	2-12-88	single, beg 96 hr post	ip	50-400	400	?		
3588	Meta Fluoro ABPP	318	3-18-88	single, beg 4 hr pre	ip	50-400	400	±	<50	
3588	Meta Fluoro ABPP	319	3-18-88	single, beg 4 hr post	ip	50-400	400	+	100	
3588	Meta Fluoro ABPP	320	3-18-88	single, beg 24 hr post	ip	50-400	400	+	50	
3588	Meta Fluoro ABPP	321	3-18-88	single, beg 48 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	322	3-18-88	single, beg 72 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	323	3-18-88	single, beg 96 hr post	ip	50-400	400	-	<50	
3588	Meta Fluoro ABPP	344	4-22-88	single, beg 4 hr pre	ip	37.5-300	300	±	75	

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3588	Meta Fluoro ABPP	345	4-22-88	single, beg 4 hr post	ip	37.5-300	300	±	75	
3588	Meta Fluoro ABPP	346	4-22-88	single, beg 24 hr post	ip	37.5-300	300	+	37.5	
3588	Meta Fluoro ABPP	347	4-22-88	single, beg 48 hr post	ip	37.5-300	300	-	>300	
3588	Meta Fluoro ABPP	348	4-22-88	single, beg 72 hr post	ip	37.5-300	300	-	>300	
3589	5-Chloro-2,3-difluorophenyl ABPP	124	9-3-87	qd x 3, beg 24 hr pre	ip	50-400	>400	+	200	
3589	5-Chloro-2,3-difluorophenyl ABPP	125	9-3-87	single, beg 24 hr pre	ip	50-400	400	-	>400	
3589	5-Chloro-2,3-difluorophenyl ABPP	176	10-29-87	qd x 3, beg 24 hr pre	ip	50-400	>400	±	400	BALLIET
3589	5-Chloro-2,3-difluorophenyl ABPP	458	9-7-88	qd x 3, beg 24 hr pre	po	31.3-500	>500	±	250	EXPANDED
3593	Ly 253,963	389	6-2-88	tid x 6, beg 24 hr pre	ip	1.2-150	>150	-	>150	
3593	Ly 253,963	390	6-2-88	bid x 6, beg 24 hr pre	ip	1.2-150	>150	-	>150	
3593	Ly 253,963	459	9-8-88	single, 24 hr pre	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	460	9-8-88	single, 4 hr post	ip	31.3-500	>500	±	31.3	
3593	Ly 253,963	461	9-8-88	single, 24 hr post	ip	31.3-500	>500	±	31.3	
3706	Tiazofurin triacetate	499	10/19/88	ad lib x 7, beg 4 hr pre drink water	po	0.96-93	>93	±	0.96	EXPANDED
3706	Tiazofurin triacetate	301	3-4-88	bid x 5, beg 4 hr pre	sc	56.3-450	>450	+	225	
3706	Tiazofurin triacetate	405	6-17-88	bid x 5, beg 4 hr pre	sc	75-600	>600	+	75	EXPANDED
3706	Tiazofurin triacetate	456	9-8-88	bid x 5, beg 24 hr pre	ip	100-800	~800	-	>800	BALLIET
3925	du Pont A2222-1	529	11/10/83	bid x 5, beg 4 hr pre	po	43.8-700	>700	+	175	EXPANDED
3925	du Pont A2222-1	189	11-12-87	single, beg 24 hr pre	ip	25-200	50	-	100	
3925	du Pont A2222-1	219	12-11-87	single, beg 4 hr pre	ip	25-200	50	-	>200	
3925	du Pont A2222-1	220	12-11-87	single, beg 4 hr post	ip	25-200	50	±	25	
3925	du Pont A2222-1	221	12-11-87	single, beg 24 hr post	ip	25-200	50	-	>200	
3925	du Pont A2222-1	222	12-11-87	single, beg 48 hr post	ip	25-200	50	-	>200	
3925	du Pont A2222-1	275	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	25	?		
3925	du Pont A2222-1	300	3-4-88	single, beg 4 hr pre	ip	3.13-25	>25	-	>25	
3925	du Pont A2222-1	406	6-15-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	±	6.25	
3925	du Pont A2222-1	441	7-20-88	3 times, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3925	du Pont A2222-1	442	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3925	du Pont A2222-1	530	11/9/88	single, beg 4 hr pre	ip	6.25-200	>200	+	6.25	EXPANDED
3925	du Pont A2222-1	531	11/10/88	single, beg 4 hr pre	ip	6.25-200	>200	-	>200	EXPANDED
3926	du Pont A2227-1	190	11-12-87	single, beg 24 hr pre	ip	25-200	25	-	25	
3926	du Pont A2227-1	223	12-11-87	single, beg 4 hr pre	ip	25-200	25	-	>200	
3926	du Pont A2227-1	224	12-11-87	single, beg 4 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	225	12-11-87	single, beg 24 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	226	12-11-87	single, beg 48 hr post	ip	25-200	25	-	>200	
3926	du Pont A2227-1	276	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3926	du Pont A2227-1	421	6-30-88	single, beg 24 hr pre	ip	12.5-100	>100	±	25	
3926	du Pont A2227-1	422	6-30-88	single, beg 4 hr pre	ip	12.5-100	>100	+	25	
3927	du Pont A754-1	443	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3927	du Pont A754-1	191	11-12-87	single, beg 24 hr pre	ip	25-200	100	-	100	
3927	du Pont A754-1	227	12-11-87	single, beg 4 hr pre	ip	25-200	200	-	>200	
3927	du Pont A754-1	228	12-11-87	single, beg 4 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	229	12-11-87	single, beg 24 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	230	12-11-87	single, beg 48 hr post	ip	25-200	200	-	>200	
3927	du Pont A754-1	277	2-10-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3927	du Pont A754-1	315	3-16-88	qd x 5, beg 36 hr pre	ip	3.13-25	>25	?		
3927	du Pont A754-1	341	4-22-88	qd x 5, beg 24 hr pre	ip	3.13-25	>25	-	>25	
3927	du Pont A754-1	411	6-24-88	bid x 5, beg 24 hr pre	ip	3.13-25	>25	-	>25	

AVS #	Compound Name	Expt. #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
3927	du Pont A754-1	423	6-30-88	single, beg 24 hr pre	ip	25-200	>200	-	>200	
3927	du Pont A754-1	424	6-30-88	single, beg 4 hr pre	ip	25-200	>200	±	200	
3927	du Pont A754-1	444	7-20-88	bid x 5, beg 24 hr pre	ip	2.5-40	>40	-	>40	
3933	Ge 089	303	3-3-88	qd x 5, beg 24 hr pre	ip	31.3-250	>250	-	>250	
3934	Ge 132, Germanium	192	11-12-87	qd x 7, beg 24 hr pre	po	9.4-300	>300	±	9.4	
3934	Ge 132, Germanium	218	12-10-87	qd x 7, beg 24 hr pre	ip	18.8-300	300	±	300	
3934	Ge 132, Germanium	367	5-6-88	bid x 7, beg 24 hr pre	ip	37.5-300	>300	+	37.5	
3934	Ge 132, Germanium	368	5-6-88	bid x 7, beg 4 hr pre	ip	37.5-300	>300	+	37.5	
3934	Ge 132, Germanium	387	6-3-88	bid x 5, beg 4 hr pre	ip	4.7-300	>300	±	4.7	EXPANDED
3934	Ge 132, Germanium	388	6-3-88	bid x 7, beg 4 hr pre	po	4.7-300	>300	±	18.8	EXPANDED
3934	Ge 132, Germanium	485	10/5/88	bid x 7, beg 24 hr pre	ip	18.8-600	>600	-	>600	EXPANDED
3934	Ge 132, Germanium	486	10-5-88	bid x 7, beg 24 hr pre	po	18.8-600	>600	-	>600	EXPANDED
3934	Ge 132, Germanium	487	10-5-88	bid x 7, beg 48 hr pre	po	18.8-600	>600	±	75	EXPANDED
3934	Ge 132, Germanium	515	10/26/88	single, beg 24 hr pre	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	516	10/27/88	single, beg 4 hr post	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	517	10/27/88	single, beg 24 hr post	ip	18.8-300	>300	-	>300	
3934	Ge 132, Germanium	542	11/22/88	single, beg 4 hr post	ip	100-800	>800	±	100	BALLIET
3934	Ge 132, Germanium	555	12/6/88	tid x 7, beg 48 hr pre	po	4.7-600	>600	±	37.5	EXPANDED
3960	DMG	196	11-19-87	bid x 7, beg 36 hr pre	po	6.3-800	>800	-	>100	
3960	DMG	197	11-19-87	bid x 7, beg 36 hr pre	sc	6.3-800	>800	-	>100	
3960	DMG	279	2-11-88	bid x 7, beg 24 hr pre	ip	9.4-600	>600	?		
3960	DMG	349	4-22-88	bid x 5, beg 24 hr pre	sc	112.5-900	>900	-	>900	
4113	Pseudolycorine HCl	433	7-14-88	qd x 5, beg 4 hr pre	sc	0.75-12	>12	±	0.75	
4282	AM-5	463	9-14-88	single, beg 24 hr pre	ip	12.5-200	12.5	-	<12.5	
4282	AM-5	464	9-14-88	single, beg 4 hr post	ip	3.125-50	3.125	-	3.125	
4282	AM-5	465	9-14-88	single, beg 24 hr post	ip	3.125-50	3.125	-	3.125	
4282	AM-5	494	10-12-88	single, beg 24 hr pre	ip	0.025-0.8	all lost wt.	-	0.05	
4282	AM-5	495	10-12-88	single, beg 4 hr post	ip	0.025-0.8	all lost wt.	±	0.025	
4282	AM-5	496	10-12-88	single, beg 24 hr post	ip	0.025-0.8	all lost wt.	+	0.025	
4282	AM-5	552	12/1/88	single, beg 24 hr post	ip	0.025-0.8	0.4	+	0.025	EXPANDED
4282	AM-5	553	12/1/88	single, beg 48 hr post	ip	0.025-0.8	0.4	±	0.2	EXPANDED
4283	AM-6	466	9-14-88	single, beg 24 hr pre	ip	12.5-200	all lost wt.	+	12.5	
4283	AM-6	467	9-14-88	single, beg 4 hr post	ip	12.5-200	all lost wt.	±	25	
4283	AM-6	468	9-14-88	single, beg 24 hr post	ip	12.5-200	all lost wt.	+	12.5	
4284	AM-7	469	9-14-88	single, beg 24 hr pre	ip	11.25-80	>180	+	11.25	
4284	AM-7	470	9-14-88	single, beg 4 hr post	ip	11.25-80	>180	-	>180	
4284	AM-7	471	9-14-88	single, beg 24 hr post	ip	11.25-80	>180	±	22.5	
4285	AM-8	472	9-14-88	single, beg 24 hr pre	ip	6.25-100	>100	-	>100	
4286	P-136	488	10-5-88	single, beg 24 hr pre	ip	12.5-200	>200	+	12.5	
4286	P-136	489	10-5-88	single, beg 4 hr post	ip	12.5-200	>200	+	25	
4286	P-136	490	10-5-88	single, beg 24 hr post	ip	12.5-200	>200	+	12.5	
4287	P-117	478	9-21-88	single, beg 24 hr pre	ip	12.5-200	all lost wt.	+	12.5	
4287	P-117	479	9-21-88	single, beg 4 hr post	ip	12.5-200	all lost wt.	+	25	
4287	P-117	480	9-21-88	single, beg 24 hr post	ip	12.5-200	all lost wt.	+	12.5	
4287	P-117	504	10/27/88	single, beg 24 hr post	ip	0.78-50	>50	+	0.78	EXPANDED
4593	P-188	482	9-29-88	single, beg 24 hr pre	ip	12.5-200	>200	+	12.5	INITIAL
4593	P-188	483	9-29-88	single, beg 4 hr post	ip	12.5-200	>200	±	12.5	INITIAL
4593	P-188	484	9-29-88	single, beg 24 hr post	ip	12.5-200	>200	±	12.5	INITIAL

AVS #	Compound Name	Expt. #	Test Date	Treatment Schedule	Route	Dose Range	Tox. @	Results	MIC	Remarks
4616	Noxymethyl pennicillinic acid	412	6-24-88	bid x 5, beg 4 hr pre	sc	18.8-150	>150	-	>150	INITIAL
4726	CPG 19835 A Lipid - Placebo	462	9-8-88	single, beg 24 hr post	ip	undilute	no	-	>undilute	EXPANDED
01 + 2149	Ribavirin + Ampligen	163	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 5	+	0.32 + 5	COMBINATION
01 + 2149	Ribavirin + Ampligen	164	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 0.5	+	0.32 + 0.5	COMBINATION
01 + 2149	Ribavirin + Ampligen	165	10-16-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150 + 0.05	+	0.32 + 0.05	COMBINATION
01 + 2149	Ribavirin + Ampligen	194	11-13-87	01 bid 2149 qd x 5, 24 hr post	po, ip		>150+0.005	+	0.32 + 0.005	COMBINATION
206 + 2776	Ribamidine + Bropirimine	288	2-19-88	206 bid x 5 2776 single, 24 post	po	2.4-75, 100	>75 + 100	+	2.4 + 100	COMBINATION
206 + 2776	Ribamidine + Bropirimine	289	2-19-88	206 bid x 5 2776 single, 24 post	po	2.4-75, 50	>75 + 50	+	2.4 + 50	COMBINATION
206 + 2776	Ribamidine + Bropirimine	290	2-19-88	206 bid x 5 2776 single, 24 post	po	2.4-75, 25	>75 + 25	+	2.4 + 25	COMBINATION
206 + 1767	Ribamidine + AM-3	383	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2.4-75, 50	>75 + 50	+	4.7 + 50	COMBINATION
206 + 1767	Ribamidine + AM-3	384	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2.4-75, 16	>75 + 16	+	4.7 + 16	COMBINATION
206 + 1767	Ribamidine + AM-3	385	5-27-88	206 bid x 5 1767 single, 48 post	po, sc	2.4-75, 5	>75 + 5	+	37.5 + 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	428	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 5	>200 + 5	+	1.0 + 5	COMBINATION
01 + 1754	Ribavirin + MVE-2	429	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 0.5	>200 + 0.5	+	1.0 + 0.5	COMBINATION
01 + 1754	Ribavirin + MVE-2	430	7-7-88	01 bid x 5, 1754 single, 24 post	po, ip	1-200 + 0.05	>200 + 0.05	+	32 + 0.05	COMBINATION

V. EFFECT OF AVS COMPOUNDS ON HEPATOTROPIC INFECTIONS IN MICE INDUCED BY THE ADAMES STRAIN OF PUNTA TORO VIRUS

Introduction

This report describes initial experiments run to determine if new AVS compounds submitted to us were active vs the hepatotropic PTV. The initial evaluation of potential anti-PTV compounds is performed using death only as endpoint. Compounds found positive in this initial evaluation are then retested using expanded evaluation parameters. If the compound is negative after the initial evaluation, further tests using other treatment regimens may be run in consultation with our Contracting Officer's Technical Representative. Figures V-1 and V-2 show flow charts for our *in vivo* evaluation process.

Materials and Methods

Virus: The Adames strain of PTV was used. This was identified as virus pool #215588 by Dr. D. Pifat of the USAMRIID, and had been safety tested by Dr. Pifat prior to being sent to us. This was a twice-plaque isolated virus prepared in LLC-MK_2 cells. The experiments run during this third year of the project used a new, more lethally potent PTV obtained by using low multiplicity of infection coupled with late harvest of infected supernate as described in Section I of our Report No. 2.

Animals: Three week-old C57BL/6 mice were obtained from Simonsen Laboratories (Gilroy, CA). All were quarantined 24 to 48 hr prior to use and maintained on Wayne Lab Blox mouse chow and tap water ad libitum. Female mice were used in all antiviral experiments and caged 10 to a cage; males were used for toxicity controls and held 5 to a cage.

Compounds: All compounds were submitted to us by Technassociates, Inc. Compounds were usually prepared one day prior to being used for the first time in an experiment, using the vehicle considered most appropriate. Insoluble compounds were subjected to 15-30 min. treatment in a sonifying water bath, warmed to 45°C, vortexed, and used as a suspension if a full solution was not achieved. Each was distributed to sterile injection bottles, sealed and stored at 4°C until used. During use, each was stored at room temperature unless we were advised to the contrary. 1-ß-D-Ribofuranosyl-1,2,4-triazole-3-carboxamide (ribavirin, AVS01) was included in each series of experiments as a known positive control.

Experiment Design: A total of 10 s.c.-infected mice were treated with each drug dosage, and 20 infected mice were treated with placebo (drug vehicle) as virus controls. Five sham-infected mice were used in each drug dosage as toxicity controls, and 5 or 10 additional mice were used as normal controls. The toxicity and normal controls were held in a room separate from the infected area. Treatments were s.c., b.i.d. x 5 beginning 4 hr pre-virus inoculation unless another treatment schedule was recommended to us by the COTR or other individual acquainted with the material to be tested. Because of the pretreatments, the animals could not be randomized after virus infection, but the infection was given to each cage on a random, scattered basis in an attempt to randomize between cages. The animals were examined daily for death through day 21. Toxicity and normal controls were weighed on day 0 and again 18 hr after final drug treatment to ascertain weight loss or failure to gain weight. Dosages ranged in 2-fold dilutions, the number of dosages depending on the compound and what was initially known about it. A single dose of ribavirin was run in parallel as a positive control. This compound was described previously by us (Sections VIII-X of Report No. 1).

In follow-up studies to confirm initial antiviral activity seen, or when oral therapy was employed, the infection parameters were extended to include reduction in hepatic icterus (liver score assigned a reading of 0, or normal, to 4, or maximum discoloration), serum glutamic oxaloacetic and pyruvic acid transaminases (SGOT, SGPT), recoverable virus from liver and from serum of infected animals 3 or 4 days after virus inoculation. Titration of SGOT and SGPT was accomplished by using colorimetric kits from Sigma Chemical Co. (St. Louis, MO). Spectrophotometric readings for these colorimetric assays were performed in duplicate by using a microplate autoreader (EL309, Bio-Tek Instruments, Inc., Winooski, UT). Livers were homogenized to a 10% (wt/vol) suspension prepared in minimum essential medium (MEM); liver homogenates and serum samples were assayed for PTV by diluting each 10-fold to a titer of 10^{-5}; 0.2 ml of each dilution were added to triplicate cups of LLC-MK_2 cell monolayers in 96-well

microplates. Viral CPE was determined after 5 days incubation at 37°C, and 50% endpoints determined (1).

Statistical Analysis: Increases in survivors were analyzed using chi-square analysis with Yates correction. Increases in mean survival times of mice that died on or before day 21 and reductions in SGOT, SGPT and PTV levels in liver or serum were evaluated using Student's *t* test. Ranked sum analysis (Wilcoxon test) was used to compare inhibition of mean liver scores.

Results and Discussion

Section IV of this report (Table IV-1) is an overview summary of all *in vivo* anti-PTV experiments run to date since the beginning of this contract in December, 1985, including those using the Balliet strain of virus described in more detail in Section VI. Tables V-1 through V-166 show the individual experiments for all compounds evaluated during this contract year.

AVS206 (Ribavirin triacetate) (Tables V-1—V-4): This compound continues to exhibit strong activity against PTV infection, with a TI at least equivalent to ribavirin. The compound is about 8 to 10 times more tolerated than ribavirin but the MIC is also higher. During this year, single or bid x 5 p.o. therapy given as late as 48 hr after virus inoculation was highly effective as seen by essentially all parameters. We must stress that toxic doses have not yet been reached because of an inadequate supply of the compound. We consider AVS02 and AVS206 to be approximately equal in activity.

AVS52 (Thioformycin B) (Tables V-5—V-6): This compound has strong *in vitro* anti-PTV activity, but only moderate *in vivo* efficacy which is highly dependent on treatment schedule, with tid or qid therapy necessary for activity to be seen.

AVS79 (9-ß-D-Ribofuranosyl purine -3-thiocarboxamide) (Tables V-7—V-11): We had previously reported AVS79 to be quite active vs PTV when given s.c. beginning 48 hr after virus inoculation. Oral therapy during this year was only moderately effective irregardless of when treatment was begun. Single p.o. therapy, especially given at 48 hr post-PTV inoculation, was also found to be highly active (Table V-10). Delaying therapy to 60 hrs after virus exposure was ineffective (Table V-11).

AVS111 (Tiazofurin) (Table V-12): Tiazofurin was previously found to be quite active vs PTV, but probably to a lesser extent than ribavirin. A single treatment run this year showed p.o. therapy to be quite effective, but again the TI, considered to be approximately 4-8, was less than ribavirin.

AVS147 (Enviroxime) (Tables V-13—V-15): Single s.c. treatments with AVS147 were previously found moderately effective vs PTV infections. Oral therapy, run during this year, was considered less effective than s.c. treatment.

AVS167 (Glycerrhetic acid) (Table V-16): Previously we found that bid therapy was not effective vs PTV using AVS167. Treatment tid run during this report period also failed to affect the infection.

AVS206 (Ribamidine, ribavirin carboxamidine) (Tables V-17—V-18): Our previous work with this compound has led to the conclusion that it is a highly active anti-PTV material and one of the best candidates found so far for further evaluation. The material has been consequently used in combination chemotherapy studies and against the Balliet virus infection as discussed elsewhere in this report. Two experiments were run to determine accurate TI's using AVS206 s.c. or p.o. A TI of 16 was found.

AVS215 (3-Deazaguanosine) (Table V-19): This is a closely related derivative of AVS272, 3-deazaguanine, which was found previously to be quite active vs PTV. In the single study run to date with AVS215, only slight activity was seen, other treatment regimens will be evaluated.

AVS222 (3-Bromo-2-chloropyrazolo-[3,4-d]-pyrimidine) (Tables V-20—V-23): Weak activity vs PTV was seen previously with AVS222. In a series of single i.p. treatment studies as well as a tid therapy experiment, only weak activity was seen.

AVS257 (Tiazofurin-5'-monophosphate) (Tables V-24—V-25): Initial studies with this compound indicate it to have significant activity vs PTV when administered i.p. on a bid treatment schedule. All infection parameters were inhibited and the material was well tolerated at all dosages used.

AVS272 (3-Deazaguanine) (Tables V-26—V-29): Twice daily treatments with this compound were ineffective vs PTV; once daily treatment was significantly inhibitory to the infection in one experiment but not in a second. Oral treatment was only weakly effective.

AVS361 (Pancratistatin) (Table V-30): In the single experiment run with this compound, no anti-PTV activity was seen.

AVS1754(MVE-2) (Tables V-31—V-34): This known immunomodulator was markedly inhibitory to PTV in our previously reported studies. This observation was followed up to show qd x 3 or bid x 5 i.p. therapy highly effective; once only s.c. treatment was as effective as similar therapy i.p. Late single treatments starting as late as 72 or 96 hr after virus inoculation were not inhibitory to the disease.

AVS1761 (Poly IC•LC) (Tables V-35—V-38): This known IFN inducer was highly active vs PTV infections. Once daily i.p. or s.c. treatments for 8 days markedly inhibited the disease, but similar p.o. therapy (Table V-37) was ineffective. Single i.p. treatments started as late as 48 hr after PTV exposure were also acceptably active.

AVS1767 (AM-3) (Tables V-39—V-42): This immune modulator was previously reported by us to be highly effective vs PTV when given s.c. bid x 5. Single s.c. treatments and s.c. once daily for 5 days were markedly inhibitory given as late as 72 hr after virus exposure at a single tolerated dose (Table V-41). Treatments i.p. bid x 5 were also effective.

AVS1778 (Mannozym) (Tables V-43—V-48): This immune modulating substance was found previously to be active vs PTV. An experiment run to repeat our earlier findings failed to demonstrate anti-PTV effects when the material was given s.c. in a single injection (Table V-43) using a new lot of the compound. Using the old lot, activity was again seen (Table V-46). Other experiments run with new material sent to us showed mannozym to be effective s.c. on a qd x 5 schedule but not on a bid x 5 schedule (Tables V-44, V-45, V-47). A later bid x 5 s.c. experiment showed strong activity, however. We are puzzled by the erratic activity of this compound, and suspect it may be labile to extensive handling and storage. Further experiments will be run with it.

AVS1969 (CL259,763) (Tables V-49—V-56): This is an immune modulator described at a recent meeting as highly active vs several *in vivo* virus infections when used orally. In our hands, p.o. therapy given once only at varying times relative to virus inoculation or bid x 5 were only weakly effective (Tables V-49—V-53, V-56). Single i.p. treatment was moderately more inhibitory (Table V-54), but i.p. therapy bid x 5 was only weakly effective (Table V-55).

AVS1976 (Thymine riboside 2',3'-dialdehyde) (Tables V-57—V-59): This compound was considered only weakly effective vs PTV, with best effects seen using single i.p. treatments (Table V-58).

AVS2149 (Ampligen, poly I•poly C12u) (Tables V-60—V-66). This known IFN inducer was previously found by us to be highly active vs *in vivo* PTV infections. We have further evaluated this material to determine influence of treatment schedule, with qd x 5, bid x 5, or single i.p. treatments being significantly inhibitory. The single treatments could be given as late as 48 hr but not 72 hr after PTV exposure. The AVS2149 effects were not influenced by size of virus inoculum (Table V-66).

AVS2700 (6-Ethylthiopurine riboside) (Tables V-67—V-69): This newly submitted material was highly active vs PTV in the initial experiment run with it. A second and third experiment failed to confirm this activity. This material was supplied to us in 2 vials; the first test used one vial, whereas the 2nd test (Table V-68) used a mixture of the two vials, with most of the compound coming from the 2nd vial. This 2nd test showed activity at a single high dose only. The third, totally inactive test, used the 2nd vial only. We conclude two compounds were sent to us, with that in vial #1 being active.

AVS2712 (Bryostatin 1) (Tables V-70—V-74): This material was considered weakly effective vs PTV using a qd x 5 treatment regimen. Once only i.p. therapy was considered more effective, with activity seen at widely different dosages, suggesting a duo mechanism of action or an immunomodulating effect with the compound.

AVS2713 (Bryostatin 2) (Tables V-75, V-76): No activity was seen in two anti-PTV experiments run with this compound. Since it is apparently related to AVS2712, we feel a single treatment should also be attempted if more compound can be provided.

AVS2741 (1-(ß-D-Ribofuranosyl)-1,2,4-triazole-3-(1,4,5,6-tetrahydropyrimidine)•HCl) (Table V-77): Weak activity was seen vs PTV in the single experiment run with this compound. It was well tolerated at all dose levels, indicating a need for higher doses to be used when more material can be made available to us.

AVS2776 (Bropirimine, ABPP, 2-Amino-5-bromo-6-phenyl-4(3H)-pyrimidinone) (Tables V-78—V-83): Bropirimine, an orally effective immunomodulator, has previously been shown by us to be highly active vs *in vivo* PTV infections. Further work done this year showed p.o. treatment qd x 3 or once only and s.c. treatment qd x 3 to be highly effective. The efficacy of the material was not significantly affected by increasing virus dosage (Tables V-82, V-83). We consider this compound one of the best anti-PTV compounds evaluated to date.

AVS2777 (AIPP, 2-Amino-5-iodo-6-phenyl-4(3H)-pyrimidinone) (Tables V-84, V-85): This immunomodulator was previously shown by us to be active vs PTV but inferior to its chemical relative, AVS2776. Our studies using this material by p.o. treatment further indicates it is active but to a lesser degree than AVS2776.

AVS2778 (ABMP, 2-Amino-5-bromomethyl-4(3H)-pyrimidinone) (Tables V-86—V-88): This immunomodulator was previously shown by us to have positive anti-PTV activity. In this year, single i.p. treatments were highly effective. Oral therapy qd x 3 were also efficacious, but to a lesser degree than the related AVS2776.

AVS2779 (MVE-1) (Table V-89): This immunomodulator was earlier reported by us to be highly effective vs PTV infections. In the one experiment run this year, single i.p. treatments were markedly inhibitory when given as late as 24 hr after PTV exposure.

AVS2811 (7-Deoxynarciclasine) (Tables V-90, V-91): This material had significant *in vitro* anti-PTV activity; used i.p. qd x 5, no efficacy was seen. However, i.p. treatment bid x 5 was effective at a single dosage level.

AVS2812 (Narciclasine) (Tables V-92, V-92B): Narciclasine had positive *in vitro* anti-PTV activity, and in a single experiment exhibited significant effects against the PTV disease *in vivo* as well. A second experiment confirmed this positive activity.

AVS2880 (Oxamisole) (Tables V-93—V-100): This immunomodulator has previously exhibited erratic *in vivo* anti-PTV activity. Other treatment regimens were studied during this report period, but none were especially effective. We conclude oxamisole to be a weak anti-PTV agent.

AVS2933 (CGP 19835, liposome monomer tripeptide) (Tables V-101—V-103): This immunomodulator was considered significantly active vs PTV infections, especially if given i.p. in a single treatment 24 hr after PTV inoculation.

AVS2978 (Tetracetate ester of AVS2980) (Tables V-104, V-105): This material exhibited an erratic anti-PTV effect in the first experiment run with it; the activity could not be confirmed in a later study. We have no more material so further evaluation of this compound cannot be done.

AVS2980 (Tetrahydroxy analogue of pancratistatin) (Tables V-106—V-108): This material was moderately active *in vitro* vs PTV, but three *in vivo* studies with it failed to show positive effects.

AVS3425 (8-Bromoguanosine) (Tables V-109—V-114): This material was considered only marginally effective vs PTV *in vivo*, although single s.c. treatments, especially given 4 hr after virus inoculation, had significant infection inhibitory effects at 2 dosage levels.

AVS3580 (Unidentified) (Tables V-115, V-116): This compound was considered marginally effective vs *in vivo* PTV in the two experiments run with it.

AVS3585 (ACPP, 2-Amino-5-chloro-6-phenyl-4(3H)-pyrimidinone) (Tables V-118—V-120): We previously showed this compound to be active vs PTV, but to a lesser extent than the structural relative, ABPP (AVS2776). Further work with it this year indicated it to be only weakly effective orally and overall less efficacious than AVS2776.

AVS3588 (Metafluoro derivative of ABPP) (Tables V-121, V-122): This material was previously shown by us to have strong efficacy vs PTV *in vivo*. This observation was extended this year to show single i.p. therapy as late as 24 hr post-virus inoculation to be effective. AVS2776, the chemical relative, was effective up to 48 hr after PTV inoculation.

AVS3589 (2-Amino-5-chloro-2,3-difluorophenyl-4(3H)-pyrimidinone) (Table V-123): This immune modulator, a derivative of AVS2776, was previously found by us to be active but possibly inferior to the latter material. Oral therapy in this report period indicated it to be much less effective than AVS2776.

AVS3593 (Ly 253,963) (Tables V-124—V-127): This immune modulator was considered only weakly inhibitory to PTV in four experiments run to date with it.

AVS3706 (Tiazofurin triacetate) (Tables V-128, V-129): This compound was considered highly active vs *in vivo* PTV infections in two experiments run to date with it. We are awaiting an additional supply of the compound.

AVS3925 (du Pont A2222-1) (Tables V-130—V-135): We previously reported this material to be inactive but possibly toxic due to the DMSO/Tween 80 vehicle used. The material appeared significantly active vs PTV especially when used in CMC vehicle in a single i.p. therapy prior to virus exposure.

AVS3926 (du Pont A2227-1) (Tables V-136—V-138): This compound, like AVS3925, was most effective vs PTV when used in CMC vehicle in a single i.p. injection.

AVS3927 (du Pont A754-1) (Tables V-139—V-143): See the comments for AVS3925, which also apply to this material. Again, single i.p. treatments were most effective, but this material appeared weaker in antiviral activity than either AVS3925 or 3926.

AVS3933 (Ge 089) (Table V-144): No activity was seen in the single experiment run with this compound. The material was nontoxic at all doses used, so should be repeated if a new supply can be provided.

AVS3934 (Ge 132) (Tables V-145—V-152): We have run a variety of experiments with Ge 132 in an attempt to accurately define its *in vivo* anti-PTV activity. The results have been confused in one may, however, in that a dry substance was initially provided to us, but later a liquid material was substituted. Treatments i.p. qd x 7 or bid x 7 appeared effective (Tables V-145, V-146) when begun prior to PTV exposure. Shortening the i.p. therapy to 5 days lessened the efficacy (Table V-147). Treatments p.o. bid x 7 were also less efficacious (Table V-148). The liquid form of this material was not effective when the i.p. bid x 7 treatment was repeated (Table V-149). Oral gavage treatments bid x 7 beginning 48 hr prior to virus exposure improved the material's antiviral effects (Table V-151).

AVS3960 (DMG) (Tables V-153—V-155): No activity was seen in 3 experiments run with this compound, but the material needs further evaluating using higher dosage levels.

AVS4113 (Pseudolycorine•HCl) (Table V-156): No activity was seen vs PTV in the single experiment run with up, but all dosages used appeared well tolerated, indicating a need to repeat the study with higher dosages.

AVS4282 (AM-5) (Tables V-157, V-158): This immunomodulator was highly active vs PTV in the only acceptable experiment run with it, with the greatest inhibitory effects seen when it was administered in a single i.p. injection 24 hr after virus inoculation.

AVS4283 (AM-6) (Table V-159): This immunomodulator was quite effective vs PTV in the single experiment run with it, with greatest activity seen in a single i.p. injection given 24 hr after virus inoculation.

AVS4284 (AM-7) (Table V-160): this immunomodulator was inactive vs PTV, but was run in a single injection 24 hr pre-PTV inoculation. If this material behaves like AVS4282, 4283, and 4284, better activity will be seen if it is given later in the infection. We are awaiting a new supply of the compound.

AVS4286 (P-136) (Table V-162): Strong anti-PTV activity was seen when this apparent immune modifier was given in a single injection as late as 24 hr after virus exposure.

AVS4287 (P-117) (Tables V-163, V-164): This immunomodulator was markedly effective vs PTV in two experiments run to date. Efficacy was most pronounced when given late (24 hr post) in the infection.

AVS4593 (P-188) (Table V-165): Relatively strong anti-PTV activity was seen with this immune modifier, especially when given i.p. in a single injection 24 hr post-virus exposure.

AVS4616 (Noxymethyl pennicillinic acid) (Table V-160): No activity was seen in the single experiment run with this compound.

Conclusions

A total of 275 *in vivo* anti-PTV experiments have been run with 56 AVS compounds using the Adames strain of PTV. Promising compounds include AVS02, 111, 206, 257, 272, 1754, 1761, 1767, 1778, 1969, 2149, 2700, 2776, 2778, 2779, 2811, 2812, 2933, 3587, 3588, 3706, 3925, 3926, 3934, 4282, 4283, 4286, 4287, and 4593, which warrant further studies with them.

Table V-1. Expt. PtA339. Effect of Per Os Once Daily Treatment with AVS02 Given 24 hr After Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.9-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS02	500	5/5	0.6	10/10**	>21.0**	0.3**	10/10**(90**)	10/10**(60**)	3.7**	3.7**
	250	5/5	0.7	7/10**	9.0**	0.5**	8/10**(276**)	2/10(291**)	3.5**	4.2**
	125	5/5	0.7	4/10*	7.8**	0.5**	2/10(427**)	1/10(520**)	3.1**	4.9**
	62.5	5/5	1.0	1/10	4.8**	0.6**	0/10(2570**)	0/10(2240**)	3.9**	5.4
Ribavirin	350	5/5	0.3	8/10**	6.5**	0.2**	10/10**(102**)	10/10**(30**)	4.0**	5.4
H_2O	-	-	-	1/20	3.7	1.5	0/18(6388)	0/18(7135)	5.6	6.0
Normals	-	5/5	0.6	-	-	0.4	5/5(78)	5/5(27)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Ribavirin triacetate was significantly active vs PTV when administered p.o. in a single treatment 24 or 48 hr after virus inoculation (PTA 339-340). The material was well tolerated at all doses given, indicating a significant therapeutic index.

*P<0.05 **P<0.01

Table V-2. Expt. PtA340. Effect of Per Os Once Daily Treatment with AVS02 Given 48 hr After Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.9-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 48 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS02	500	5/5	0.6	6/10**	6.8**	1.2	7/10**(641**)	6/10**(364**)	2.9**	2.7**
	250	5/5	0.7	2/10	5.6**	3.4	0/9(7872)	0/9(9817)	4.5**	5.8
	125	5/5	0.7	0/10	4.1	3.2	0/10(7995)	0/10(9020)	5.2	5.9
	62.5	5/5	1.0	0/10	3.9	2.8	0/10(9280)	0/10(8550)	5.2	5.7
Ribavirin	350	5/5	0.3	8/10**	6.5**	0.2**	10/10**(102**)	10/10**(30**)	4.0**	5.4
H_2O	-	-	-	1/20	3.7	1.5	0/18(6388)	0/18(7135)	5.6	6.0
Normals	-	5/5	0.6	-	-	0.4	5/5(78)	5/5(27)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: See the conclusions under PTA 339.

*P<0.05 **P<0.01

Table V-3. Expt. PtA377. Effect of Twice Daily p.o. Treatment with AVS02 on Punta Toro Virus Infections in Mice.

Animals: 12.0-14.0g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Twice daily x 5, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

	Toxicity controls			Infected-Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS02	500	5/5	1.8	10/10**	>21.0**	0.0**	10/10**(32**)	10/10**(8**)	3.5	0.0**
	250	5/5	2.4	10/10**	>21.0**	0.1**	10/10**(44**)	10/10**(9**)	1.7**	0.9**
	125	5/5	3.0	10/10**	>21.0**	0.1**	9/9**(64**)	9/9**(28**)	0.8**	1.8**
	62.5	5/5	2.3	10/10**	>21.0**	0.5**	7/10**(316**)	5/10**(368**)	3.0*	4.0*
	31.3	5/5	1.8	3/10*	7.0**	1.9	0/10(2425)	0/10(3209)	3.6	4.7
Ribavirin	75	4/5	1.3	10/10**	>21.0**	1.2	10/10**(102**)	10/10**(39**)	1.2**	3.8**
H_2O	-	-	-	0/20	4.6	1.7	1/17(3508)	1/17(3609)	4.2	5.5
Normals	-	5/5	3.6	-	-	0.0	5/5(40)	5/5(12)	1.7	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: Oral therapy with ribavirin triacetate was markedly effective vs PTV infections in this experiment. Note treatment began 24 hr post-virus inoculation in this study. The compound was well tolerated, indicating higher dosages could have been used.

*P<0.05 **P<0.01

Table V-4. Expt. PtA378. Effect of Twice Daily p.o. Treatment with AVS02 on Punta Toro Virus Infections in Mice.

Animals: 12.0-14.0g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Twice daily x 5, beginning 48 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

	Toxicity controls			Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS02	500	5/5	1.8	10/10**	>21.0**	0.4**	9/10**(610**)	8/10**(588**)	2.2**	2.8**
	250	5/5	2.4	9/10**	5.0	1.2	5/10**(998**)	2/10(780**)	3.7	3.6**
	125	5/5	3.0	7/10**	5.3	0.6**	5/10**(807**)	3/10(914**)	3.9	4.8
	62.5	5/5	2.3	9/10**	7.0	1.4	2/10(1353)	2/10(1974)	3.4	5.2
	31.3	5/5	1.8	8/9**	10.0**	3.0	0/10(5096)	0/10(5650)	2.8**	5.6
Ribavirin	75	4/5	1.3	10/10**	>21.0**	1.2	10/10**(102**)	10/10**(39**)	1.2**	3.8**
H_2O	-	-	-	0/20	4.6	1.7	1/17(3508)	1/17(3609)	4.2	5.5
Normals	-	5/5	3.6	-	-	0.0	5/5(40)	5/5(12)	1.7	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Oral therapy with ribavirin triacetate was markedly effective in this experiment vs PTV infections, with therapy begun 48 hr post-virus inoculation.

*P<0.05 **P<0.01

Table V-5. Expt. PtA231A. Effect of Four Times Daily Treatment with AVS052 on Punta Toro Virus Infections in Mice.

Animals: 10.5-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.

Treatment Schedule: 4 times daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS052	400	5/5	0.5	0/10	6.4**
	200	5/5	1.4	3/10	6.4**
	100	5/5	2.5	0/10	5.8**
	50	5/5	3.1	1/10	4.9*
	25	5/5	2.8	0/10	4.4
Ribavirin	75	5/5	2.3	9/10**	8.0
Saline	-	-	-	2/20	4.3
Normals	-	5/5	2.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS052 is thioformycin B. *In vitro* this material was highly active vs PTV, but was inactive *in vivo* using standard treatment regimens. In PtA 153, however, a thrice-daily s.c. treatment was moderately effective, suggesting the need for increased treatments per day. The present study utilized 4 x daily treatments, with positive effects seen at 4 dosage levels, the activity manifested as increased mean survival time.

Table V-6. Expt. PtA342. Effect of Per Os Thrice Daily Treatment with AVS052 on Punta Toro Virus Infections in Mice.

Animals: 10.2-11.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Three times daily x 5, 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS052^	400	5/5	1.2	1/10	5.3**	0.9	6/9**(183**)	3/9(123**)	5.1	5.2**
	200	5/5	1.8	1/10	5.0**	1.1	0/9(4793)	0/9(4806)	4.3**	6.1
	100	5/5	2.5	0/10	5.0**	0.8	2/10(1325*)	0/10(1759)	4.6*	5.8**
	50	5/5	2.9	0/10	4.8**	2.3	0/9(6443)	0/9(7639)	4.4**	6.2
Ribavirin	37.5	5/5	0.8	9/10**	8.0	0.1**	8/9**(109**)	8/9**(97**)	3.8**	3.6**
H_2O	-	-	-	0/20	4.0	0.8	1/17(3813)	2/17(3748)	5.8	6.4
Normals	-	5/5	3.9	-	-	0.0	5/5(82)	5/5(16)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Residual drug in syringes turns rusty-brown—possible reaction to plastic or metal?

Conclusions: Thioformycin B was previously found moderately active vs PTV (PTA 153) when administered s.c. by this three times daily schedule. In the present study, the material was also active, although only to a moderate degree, when administered orally by the same therapy schedule.

*P<0.05 **P<0.01

Table V-7. Expt. PtA336. Effect of Per Os Treatment Once Only with AVS079 Given 4 hr After Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 4 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS079	700	5/5	0.2	0/10	4.1	1.1	1/10(4732)	1/10(6249)	5.8	6.1
	350	5/5	0.4	0/10	4.3	0.5**	0/9(3065**)	0/9(3729**)	5.0	6.4
	175	5/5	0.4	0/10	4.1	0.5**	1/10(4497)	1/10(4696)	6.0	6.4
	87.5	5/5	0.0	0/10	3.8	1.0	0/10(6290)	0/10(6239)	6.2	6.5
Ribavirin[g]	350	5/5	0.3	6/10**	6.5**	0.2**	10/10**(102**)	10/10**(30**)	4.0**	5.4
H_2O	-	-	-	1/20	3.7	1.5	0/18(6388)	0/18(7135)	5.6	6.0
Normals	-	5/5	0.6	-	-	0.4	5/5(78)	5/5(27)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Ribavirin administered 24 hr post-virus inoculation.

Conclusions: AVS079 is 9-β-D-ribofuranosylpurine-6-thiocarboxamide. We have previously shown this material, if administered in a single s.c. injection, to be most effective when given 48 hr after virus inoculation. The present series of studies (PTA 336-338) were designed to determine if this same phenonomen was seen when the compound was given orally. As seen in PTA 338, this was indeed the case.

*P<0.05 **P<0.01

Table V-8. Expt. PtA337. Effect of Per Os Treatment Once Only with AVS079 Given 24 hr After Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS079	700	5/5	0.2	0/10	4.7**	2.2	0/10(3395**)	0/10(3920**)	5.4	6.1
	350	5/5	0.4	0/10	5.0**	1.5	0/10(2549**)	0/10(2808**)	5.2	6.0
	175	5/5	0.4	1/10	6.0**	1.7	0/10(5279)	0/10(5420)	5.3	6.1
	87.5	5/5	0.0	0/10	5.1**	2.4	0/10(7552)	0/10(7353)	5.7	6.0
Ribavirin[g]	350	5/5	0.3	8/10**	6.5**	0.2**	10/10**(102**)	10/10**(30**)	4.0**	5.4
H_2O	-	-	-	1/20	3.7	1.5	0/18(6388)	0/18(7135)	5.6	6.0
Normals	-	5/5	0.6	-	-	0.4	5/5(73)	5/5(27)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Ribavirin administered 24 hr post-virus inoculation.

Conclusions: See the conclusions in PTA 336 (Table 6).

*P<0.05 **P<0.01

Table V-9. Expt. PtA338. Effect of Per Os Treatment Once Only with AVS079 Given 48 hr After Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 48 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS079	700	5/5	0.2	2/10	4.5*	2.0	2/8*(1767**)	1/8(2322**)	3.9**	5.1**
	350	5/5	0.4	0/10	5.0**	2.4	3/10*(3294**)	2/10(3265**)	3.8**	5.7
	175	5/5	0.4	0/10	3.9	2.6	1/10(5315)	1/10(4603)	4.4**	5.9
	87.5	5/5	0.0	0/10	4.5*	2.9	0/10(7154)	0/10(8077)	4.5*	6.0
Ribavirin[g]	350	5/5	0.3	8/10**	6.5**	0.2**	10/10**(102**)	10/10**(30**)	4.0**	5.4
H_2O	-	-	-	1/20	3.7	1.5	0/18(6388)	0/18(7135)	5.6	6.0
Normals	-	5/5	0.6	-	-	0.4	5/5(78)	5/5(27)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Ribavirin administered 24 hr post-virus inoculation.

Conclusions: See the conclusions in PTA 336 (Table 6).

*P<0.05 **P<0.01

Table V-10. Expts. PtA374-376. Effect of Single p.o. Treatment with AVS079 Given at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.4-14.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS079	4 hr post	700	5/5	0.6	4/10	6.0
		350	5/5	0.8	0/10	6.0*
		175	5/5	0.8	2/10	5.1
		87.5	5/5	1.6	5/10	5.6
	24 hr post	700	5/5	0.6	1/10	6.3**
		350	5/5	0.8	3/10	5.7
		175	5/5	0.8	1/10	4.6
		87.5	5/5	1.6	0/10	4.4
	48 hr post	700	5/5	0.6	9/10**	6.0
		350	5/5	0.8	9/10**	7.0
		175	5/5	0.8	8/10**	5.0
		87.5	5/5	1.6	4/10	5.2
Ribavirin		350	5/5	1.7	10/10**	>21.0**
H_2O		-	-	-	4/20	4.9
Normals		-	5/5	1.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This experiment confirms our previous study (PtA 336-338, Tables 6-8) in which oral treatment with AVS079 (9-ß-D-Ribofuranosylpurine-6-thiocarboxamide) was most effective when given 48 hr post-virus inoculation.

Table V-11. Expt. PtA403. Effect of Once Only p.o. Treatment With AVS079 on Punta Toro Virus Infections in Mice.

Animals: 13.5-15.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 60 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS079	700	5/5	-0.3	0/10	4.8
	350	5/5	-0.2	1/10	5.2
	175	5/5	0.6	0/10	4.1
	87.5	5/5	0.1	0/10	4.2
	43.8	5/5	0.1	0/10	4.7
Ribavirin	350	5/5	0.3	2/10*	5.8
H_2O	-	-	-	0/20	4.6
Normals	-	-	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: 9-ß-D-ribofuranosylpurine-6-thiocarboxamide was previously found highly active vs PTV when administered p.o. in a single treatment given up to 48 hr post-virus exposure. In the present experiment, designed to study the effect of a 60 hr delayed treatment, no activity was seen.

Table V-12. Expt. PtA365. Effect of Twice Daily p.o. Treatment with AVS111 on Punta Toro Virus Infections in Mice.
Animals: 13.2-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

	Toxicity controls			Infected-Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS111	750	5/5	0.4	10/10**	>21.0**	0.3**	9/9**(69**)	9/9**(37**)	3.4*	4.0**
	375	5/5	1.6	7/9**	7.5**	0.6**	3/9(1726**)	1/9(2075**)	4.2	4.5**
	187.5	5/5	2.0	3/10*	4.9	1.3**	2/9(1645**)	1/9(1099**)	4.5	5.0
	93.8	5/5	2.8	0/10	4.6	2.0	1/9(6531**)	1/9(4712**)	4.9	5.5
Ribavirin	75	5/5	2.4	10/10**	>21.0**	0.0**	8/10**(160**)	10/10**(57**)	2.5**	2.4**
H_2O	-	-	-	0/20	4.0	2.6	1/19(12,487)	1/19(11,505)	4.5	6.0
Normals	-	5/5	3.6	-	-	0.0	5/5(84)	5/5(37)	2.6	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: Oral treatment with AVS111 (tiazofurin) was markedly effective vs PTV in this experiment. It appears the high dose used, 750 mg/kg/day, was approaching the MTD, since the toxicity controls at this dose gained little weight.

*P<0.05 **P<0.01

Table V-13. Expt. PtA522. Effect of Single p.o. Treatment with AVS147 24 hr Prior to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.1-15.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS147	1200	4/5	0.2	0/10	5.6	4.0	0/10(8066)	0/10(4877)	5.6	6.1
	600	5/5	0.1	1/10	5.4	3.2	0/10(5265)	0/10(3485)	5.6	5.7
	300	5/5	0.2	2/10	5.9	3.7	0/9(7971)	0/9(5289)	6.1	6.1
	150	5/5	0.5	1/10	6.2	3.4	0/9(6272)	0/9(4050)	5.5	6.0
Ribavirin	350	5/5	-0.1	9/10**	7.0	1.4**	0/10(1040**)	0/10(724**)	4.3**	4.3**
CMC	-	-	-	1/20	5.1	3.5	0/17(5227)	0/17(4720)	5.4	6.1
Normals	-	5/5	0.9	-	-	0.1	5/5(65)	5/5(19)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS147 (enviroxime) was previously found effective vs PTV in mice when administered in a single i.p. injection early in the infection (PtA 34-36). Per os therapy, give 24 hr pre-virus inoculation, which was investigated in this study, was not effective, however.

*P<0.05 **P<0.01

Table V-14. Expt. PtA523. Effect of Single p.o. Treatment with AVS147 4 hr Post-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.1-15.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS147	1200	4/5	0.2	2/10	4.4	3.4	0/10(5378)	0/10(4921)	5.7	6.1
	600	5/5	0.1	0/10	5.2	3.1	0/10(3214*)	0/10(3134*)	4.9	4.9*
	300	5/5	0.2	6/10**	5.0	3.2	0/10(4147)	0/10(4032)	5.0	5.1
	150	5/5	0.5	0/10	5.5	3.6	0/10(4457)	0/10(4262)	5.6	5.8
Ribavirin	350	5/5	-0.1	9/10**	7.0	1.4**	0/10(1040**)	0/10(724**)	4.3**	4.3**
CMC	-	-	-	1/20	5.1	3.5	0/17(5227)	0/17(4720)	5.4	6.1
Normals	-	5/5	0.9	-	-	0.1	5/5(65)	5/5(19)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS147 (enviroxime) was previously found effective vs PTV when administered i.p. 4 hr post-virus inoculation (PtA 34). Oral gavage therapy given at the same time was also somewhat effective, although not by all parameters.

*P<0.05 **P<0.01

Table V-15. Expt. PtA524. Effect of Single p.o. Treatment with AVS147 on Punta Toro Virus Infections in Mice.

Animals: 12.1-15.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS147	1200	4/5	0.2	0/10	4.3	3.6	0/10(6520)	0/10(7385)	5.5	6.1
	600	5/5	0.1	1/10	4.2	3.8	0/10(6570)	0/10(7325)	5.4	5.8
	300	5/5	0.2	1/10	4.8	3.1	0/9(6191)	0/9(6846)	5.2	5.7
	150	5/5	0.5	2/10	4.1	3.5	0/10(5[illegible]16)	0/10(5566)	4.9	5.6
Ribavirin	350	5/5	-0.1	9/10**	7.0	1.4**	0/10(1040**)	0/10(724**)	4.3**	4.3**
CMC	-	-	-	1/20	5.1	3.5	0/17(5227)	0/17(4720)	5.4	6.1
Normals	-	5/5	0.9	-	-	0.1	5/5(65)	5/5(19)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS147 (enviroxime) was previously found effective vs PTV when administered i.p. 4 hr post-virus inoculation (PtA 34). Oral gavage therapy at this same time was also moderately effective (PtA 523). Delaying the therapy to 24 hr post-virus inoculation in this experiment rendered the material not active vs PTV.

*P<0.05 **P<0.01

Table V-16. Expt. PtA304. Effect of Three Times Daily i.p. Treatment With AVS167 on Punta Toro Virus Infections in Mice.

Animals: 11.9-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Three times daily x 5, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS167	600	1/5	-2.5	0/10	4.6
	300	5/5	-0.9	0/10	4.9
	150	5/5	0.5	0/9	5.4
	75	5/5	1.2	1/10	5.0
Ribavirin	75	5/5	2.6	10/10**	>21.0**
CMC	-	-	-	0/20	5.1
Normals	-	5/5	1.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Glycerrhetic acid was previously found inactive when used bid x 5. The present study utilized a tid x 5 schedule, with the MTD being reached. No activity was seen.

Table V-17. Expt. PtA233. Effect of AVS206 on Punta Toro Virus Infections in Mice. AVS206 Lot No. 12-17-87

Animals: 10.2-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS206	2000	1/5	-2.1	0/10	6.0**
	1000	5/5	1.4	3/10*	9.9**
	500	5/5	2.3	10/10**	>21.0**
	250	5/5	2.3	10/10**	>21.0**
	125	5/5	2.0	10/10**	>21.0**
	62.5	5/5	3.1	8/10**	6.5
	31.3	5/5	3.7	2/10	4.9
	15.7	5/5	4.0	2/10	4.9
	7.8	5/5	3.5	0/10	4.1
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Saline	-	-	-	0/20	4.4
Normals	-	5/5	4.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This experiment was run to detemine an accurate therapeutic index (TI) for AVS206 (ribavirin carboxamidine) vs PTV *in vivo*. In this experiment, a more severe virus infection was reduced, so that 100% of the virus control mice died. Assuming 1000 mg/kg/day of AVS206 as the MTD, we calculate the TI to be 16. AVS206 is approximately 10 times less toxic than ribavirin, but also is less potent antiviral, since the MIC is 62.5 compared to 4.7 for ribavirin. Thus the TI's for both compounds is 16.

Table V-18. Expt. PtA234. Effect of AVS206 on Punta Toro Virus Infections in Mice. AVS206 Lot No. 12-17-87

Animals: 10.2-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS206	2000	4/5	0.8	0/10	5.6**
	1000	5/5	2.4	1/10	6.7**
	500	5/5	2.1	9/10**	12.0
	250	5/5	1.6	10/10**	>21.0**
	125	5/5	2.6	10/10**	>21.0**
	62.5	5/5	2.6	9/10**	6.0
	31.3	5/5	3.1	2/10	4.8
	15.7	5/5	1.8	3/10	5.1
	7.8	5/5	2.4	5/10*	4.6
Ribavirin	75	5/5	2.5	8/10**	8.5
Saline	-	-	-	3/20	4.0
Normals	-	5/5	4.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This experiment was run to detemine an accurate therapeutic index (TI) for AVS206 (ribavirin carboxamidine) when the compound was administered by oral gavage to PTV-infected mice. Assuming 1000 mg/kg/day to be the MTD, and 62.5 mg/kg/day to be the MIC, we calculate the TI to be 16. It is noted that 50% of the infected mice survived which were treated with the lowest dose level of AVS206; in view of no activity seen at the 15.7 and 31.3 mg/kg/day dosages, we have disregarded this result.

Table V-19. Expt. PtA497. Effect of Once Daily s.c. Treatment With AVS215 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.4-14.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS215	300	0/5	-3.8	0/10	5.6
	150	4/5	-2.2	0/10	7.1**
	75	5/5	0.3	4/10	7.3**
	37.5	5/5	2.3	3/10	6.4*
	18.8	5/5	2.3	1/10	5.7
Ribavirin	75	5/5	2.0	9/9**	>21.0**
Saline	-	-	-	4/20	5.1
Normals	-	5/5	2.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS215 (3-deazaguanosine) was moderately effective vs PTV in this initial evaluation.

Table V-20. Expt. PtA302. Effect of Once Daily s.c. Treatment With AVS222 on Punta Toro Virus Infections in Mice.

Animals: 12.0-13.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, 24 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS222	500	5/5	3.3	0/10	5.8
	250	5/5	3.0	2/10	6.1
	125	5/5	3.2	0/10	5.8
	62.5	5/5	3.2	0/10	5.1
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Saline	-	-	-	1/20	4.6
Normals	-	5/5	1.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound, 3-bromo-4-chloropyrazolo-[3,4-d]pyrimidine, was previously tested using a bid x 5 treatment schedule with no anti-PTV activity seen. In this experiment, a qd x 5 treatment schedule was also ineffective.

Table V-21. Expt. PtA366. Effect of Twice Daily s.c. Treatment With a High Dose of AVS222 on Punta Toro Virus Infections in Mice.

Animals: 11.4-13.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS222	2000	4/5^	1.5	0/10	3.8
Ribavirin	75	5/5	2.4	10/10**	>21.0**
CMC	-	-	-	0/20	4.1
Normals	-	5/5	3.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

^Toxicity controls treated twice on Day 3. One died on Day 5.

*P<0.05 **P<0.01

Conclusions: This compound, 3-Bromo-4-chloropyrazole-[3,4-d]-pyrimidine, was previously tested vs PTV using this treatment schedule using doses up to 250 mg/kg/day. The present study used a single high dose in an attempt to approach an MTD. We feel this was achieved, but no antiviral activity was seen.

Table V-22. Expt. PtA437-439. Effect of Single i.p. Treatment With AVS222 Administered at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS222	24 hr pre	1500	5/5	-0.5	0/10	5.1*
		750	5/5	-0.2	0/10	5.9**
		375	5/5	0.1	0/10	5.1**
		187.5	5/5	0.7	0/10	5.3**
	4 hr pre	1500	5/5	-0.5	0/10^	4.0
		750	5/5	-0.2	0/10	4.2
		375	5/5	0.1	0/10	4.2
		187.5	5/5	0.7	0/10	4.1
	24 hr post	1500	5/5	-0.5	0/10	4.9
		750	5/5	-0.2	0/10	3.9
		375	5/5	0.1	0/10	4.5
		187.5	5/5	0.7	0/10	4.4
Ribavirin		350	5/5	0.6	7/10**	8.3**
CMC		-	-	-	2/20	4.3
Normals		-	5/5	0.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: 3-Bromo-4-chloro-pyrazolo-[3,4-d]-pyrimidine administered 24 hr pre-virus inoculation, was effective only in moderately prolonging mean survival time in this series of experiments. We feel we approached the MTD with the 1500 mg/kg dose used in the study.

Table V-23. Expt. PtA440. Effect of Thrice Daily s.c. Treatment With AVS222 on Punta Toro Virus Infections in Mice.

Animals: 12.0-13.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Thrice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS222	500	5/5	3.2	0/10	4.1
	250	5/5	3.2	0/10	4.2
	125	5/5	3.3	0/10	4.0
	62.5	5/5	2.5	2/10	4.5
Ribavirin	75	5/5	3.0	10/10**	>21.0**
CMC	-	-	-	2/20	4.7
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: 3-Bromo-4-chloropyrazolo-[3,4-d]-pyrimidine was ineffective vs PTV when used on a tid x 5 treatment schedule. The compound was well tolerated at all dose levels.

Table V-24. Expt. PtA445. Effect of Twice Daily i.p. Treatment With AVS257 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.6-13.4 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily x 5 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS257	400	5/5	1.3	10/10**	>21.0**
	200	5/5	2.7	0/10	5.3**
	100	5/5	3.0	0/10	4.7
	50	5/5	3.3	0/10	4.1
	25	5/5	3.3	0/10	4.3
Ribavirin	75	5/5	2.9	9/10**	11.0
Saline	-	-	-	0/20	4.3
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this initial test, tiazofurin 5'-monophosphate was significantly active vs PTV. The compound was reasonably well tolerated at all dose levels, although the animals gained less weight when treated with highest dosage (400 mg/kg/day) used, suggesting the MTD was being approached.

Table V-25. Expt. PtA449. Effect of Twice Daily i.p. Treatment with AVS257 on Punta Toro Virus Infections in Mice.

Animals: 9.9-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

	Toxicity controls			Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS257	400	5/5	1.3	6/10**	5.8	0.5**	9/10**(104**)	10/10**(37**)	0.3**	1.8**
	200	5/5	3.2	9/10**	9.0	1.3**	1/10(1182**)	1/10(1307**)	3.3**	4.2**
	100	5/5	2.4	0/10	6.0**	3.5	1/10(7221)	0/10(7457)	4.8	5.6
	50	5/5	2.8	0/10	4.8	3.9	0/10(11,280)	0/10(10,790)	5.3	6.3
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.2**	8/10**(166**)	10/10**(39**)	0.3**	0.3**
Saline	-	-	-	0/20	4.2	3.7	0/20(8992)	0/20(9019)	5.0	5.5
Normals	-	5/5	3.0	-	-	0.1	5/5(116)	5/5(23)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: This is tiazofurin 5'-MP, which was shown previously (PtA445) to have strong activity vs PTV in mice. This experiment confirms and extends those initial observations. This compound was well tolerated at all dose levels used, indicating a need to use higher dosages to determine the MTD.

*P<0.05 **P<0.01

Table V-26. Expt. PtA232. Effect of Once Daily Treatments with AVS272 on Punta Toro Virus Infections in Mice.

Animals: 10.9-12.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS272	200	5/5	1.0	10/10**	>21.0**
	100	5/5	1.8	7/10**	5.0
	50	5/5	2.7	5/10	4.8
	25	5/5	3.4	8/10**	5.0
Ribavirin	75	5/5	3.3	10/10**	>21.0**
CMC	-	-	-	4/20	4.6
Normals	-	5/5	2.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS272 is 3-deazaguanine. Previous work we have done with this compound indicated it to have activity against a variety of other RNA viruses. In one previous experiment (PtA 186), twice daily treatment with AVS272 was ineffective vs PtA. In the present study, once daily s.c. treatments were effective, however, suggesting the need for further studies to elucidate the most effective treatment regimen.

Table V-27. Expt. PtA317. Effect of Twice Daily i.p. Treatment With AVS272 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS272	100	5/5	-1.2	0/10	6.8
	50	5/5	0.9	3/10	6.4
	25	5/5	0.4	0/10	5.7
	12.5	5/5	1.0	2/10	5.8
Ribavirin[c]	75	5/5	2.1	10/10	>21.0
CMC	-	-	-	1/20	6.4
Normals	-	5/5	4.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered bid x 5 beginning 4 hr pre-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This experiment was run to determine if i.p. treatment with AVS272 (3-deazaguanine) would be more effective than s.c. treatment previously found to be ineffective vs PTV. No activity was seen.

Table V-28. Expt. PtA370. Effect of Once Daily p.o. Treatment with AVS272 on Punta Toro Virus Infections in Mice.

Animals: 11.8-13.2g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] ($\log_{10}$)	Mean Serum Virus Titer[f] ($\log_{10}$)
AVS272	300	5/5	-9	1/10	6.4	0.8**	1/10(2378)	0/10(2108)	5.5	5.7
	150	5/5	-9	0/10	6.2	0.4**	1/10(2587)	1/10(2407)	4.7	4.9
	75	5/5	-9	0/10	6.5	2.4	1/10(5983)	1/10(4916)	4.0	4.6
	37.5	5/5	-9	0/10	5.8	1.8**	0/9(4594)	0/9(3533)	4.6	5.2
	18.8	5/5	-9	0/10	5.5	1.6**	1/10(3093)	1/10(3549)	5.5	5.9
Ribavirin	75	5/5	-9	10/10**	>21.0**	0.3**	10/10**(102**)	10/10**(39**)	1.2**	3.8**
CMC	-	-	-	2/20	5.6	2.8	1/16(3235)	1/16(2908)	4.4	5.5
Normals	-	5/5	-9	-	-	0.3	5/5(54)	5/5(14)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]No second weight at end of treatment.

Conclusions: Oral therapy with AVS272, 3-deazaguanine, was effective only in reducing liver scores in the PTV-infected animals.

*P<0.05 **P<0.01

Table V-29. Expt. PtA498. Effect of Once Daily s.c. Treatment with AVS272 on Punta Toro Virus Infections in Mice.

Animals: 11.5-12.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS272	300	5/5	-0.5	0/10	5.8	2.1	2/10(8605)	2/10(7356)	6.3	5.8
	150	5/5	1.3	1/9	5.0	2.1	0/10(11,543)	0/10(7754)	5.8	5.5
	75	5/5	2.0	3/10	5.3	2.9	0/10(12,416)	0/10(6441)	6.2	6.1
	37.5	5/5	2.0	0/10	6.0*	2.2	0/10(3620)	0/10(3085)	5.6	5.5
	18.8	5/5	2.9	1/10	6.6*	2.3	0/9(6695)	0/9(4854)	5.5	6.0
Ribavirin	75	5/5	2.0	9/9**	>21.0**	0.0**	7/10*(780**)	7/10*(462**)	2.7**	2.2**
CMC	-	-	-	10/20	5.2	2.6	4/18(3765)	4/18(2399)	4.8	4.6
Normals	-	5/5	2.2	-	-	0.0	4/5(282)	4/5(206)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS272 (3-deazaguanine) was previous found active vs PTV when administered i.p. by this treatment regimen (PtA 232). Treatment s.c. was not effective, however.

*P<0.05 **P<0.01

Table V-30. Expt. PtA417. Effect of Once Daily s.c. Treatment With AVS361 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.6-12.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% EtOH in saline.
Treatment Schedule: Once daily x 6, 24 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS361	4	5/5	2.9	1/10	5.9
	2	5/5	3.3	5/10	6.4
	1	4/5	2.8	5/10	6.0
	.5	5/5	3.4	3/10	6.4
Ribavirin	75	5/5	4.1	10/10*	>21.0**
Saline	-	5/5	3.4	9/20	5.0
Normals	-	5/5	4.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Pancratistatin (AVS361) was not considered effective in this experiment. The dosages and regimen used was as recommended to us. The compound appeared well tolerated in this experiment, suggesting higher dosages should be used in future studies.

Table V-31. Expt. PtA238. Effect of Once Daily Treatment with AVS1754 on Punta Toro Virus Infections in Mice.

Animals: 11.5-13.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 3, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1754	50	5/5	-0.3	10/10*	>21.0**
	25	5/5	3.0	10/10*	>21.0**
	12.5	5/5	1.5	10/10*	>21.0**
	6.25	5/5	1.7	10/10*	>21.0**
	3.13	5/5	1.6	9/10	7.0
Ribavirin	75	5/5	2.6	10/10*	>21.0**
Saline	-	-	-	14/20	5.0
Normals	-	5/5	1.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Previous work with AVS1754 (MVE-2) was all run using single i.p. treatments, with positive effects seen vs PTV *in vivo*. In the present experiment, treatements i.p. once daily for 3 days was similarly effective.

Table V-32. Expt. PtA240-241. Effects of Late Single Treatments with AVS1754 on Punta Toro Virus Infections in Mice.

Animals: 13.3-15.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once only, 72 or 96 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Hcst Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1754	72 hr post	100	5/5	0.5	6/10	4.3
		50	5/5	0.7	6/10	4.5
		25	5/5	0.3	0/10	4.6
		12.5	5/5	0.9	1/10	5.1
		6.25	5/5	0.8	6/10	5.0
	96 hr post	100	5/5	0.5	1/10	4.9
		50	5/5	0.7	2/10	5.3
		25	5/5	0.3	7/10	4.7
		12.5	5/5	0.9	4/10	5.0
		6.25	5/5	0.8	4/10	5.2
Ribavirin		350	5/5	0.4	8/10	6.0
Saline		-	-	-	14/20	5.0
Normals		-	5/5	1.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: We have previously shown AVS1754 (MVE-2) to be active vs PTV when administered in a single i.p. injection at varying times up to 48 hr post-virus inoculation. Later times of treatment were not studied because of insufficient compound. The present experiments show that delaying MVE-2 treatment to 72 or 96 hr post-virus inoculation eliminated any antiviral activity exhibited by the material.

Table V-33. Expt. PtA249. Effect of a Single s.c. Treatment with AVS1754 on Punta Toro Virus Infections in Mice.

Animals: 10.4-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
	Dosage	Surv/	Host Wt.	Surv/	MST[b]
Compound	(mg/kg/day)	Total	Change (g)[a]	Total	(days)
AVS1754	100	5/5	-0.2	10/10**	>21.0**
	50	5/5	-0.1	10/10**	>21.0**
	25	5/5	0.1	10/10**	>21.0**
	12.5	5/5	-0.5	9/10**	7.0
	6.25	5/5	0.7	6/10**	5.5
Ribavirin	350	5/5	0.2	10/10**	>21.0**
Saline	-	-	-	2/20	5.3
Normals	-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: We have previously shown that AVS1754 (MVE-2) was highly effective vs PTV when administered i.p. in single treatments at varying times relative to virus inoculation. The present experiment indicates that the material is also effective when administered s.c.

Table V-34. Expt. PtA311. Effect of Twice Daily i.p. Treatments With AVS1754 on Punta Toro Virus Infections in Mice.

Animals: 13.4-14.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1754	50	5/5	2.1	10/10**	>21.0**
	25	5/5	2.2	10/10**	>21.0**
	12.5	5/5	1.5	10/10**	>21.0**
	6.25	5/5	2.2	10/10**	>21.0**
Ribavirin	75	5/5	2.5	10/10**	>21.0**
Saline	-	-	-	1/20	4.6
Normals	-	5/5	3.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: MVE-2, used on a bid x 5 treatment schedule, was highly active vs PTV in this study.

Table V-35. Expt. PtA307. Effect of Once Daily i.p. Treatment With AVS1761 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.4-13.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 8, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1761	5	4/5	-1.4	1/10	5.2
	2.5	5/5	2.5	10/10**	>21.0**
	1.25	5/5	2.6	10/10**	>21.0**
	0.625	5/5	3.5	10/10**	>21.0**
	0.31	5/5	4.2	10/10**	>21.0**
	0.15	5/5	4.1	10/10**	>21.0**
	0.078	5/5	4.2	10/10**	>21.0**
	0.039	5/5	3.8	10/10**	>21.0**
	0.0195	5/5	4.4	8/10**	7.0
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Saline	-	-	-	3/20	5.6
Normals	-	5/5	5.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is poly IC•LC, a known interferon inducer. The material was highly active at all nontoxic dosages used in the study.

Table V-36. Expt. PtA324. Effect of Once Daily s.c. Treatment with AVS1761 on Punta Toro Virus Infections in Mice (Confirming Experiment).

Animals: 12.5-13.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 8, beginning 24 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

	Toxicity controls			Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1761	1	5/5	1.8	10/10**	>21.0**	0.1**	9/9**(78**)	9/9**(20**)	0.0**	0.0**
	0.5	5/5	3.6	10/10**	>21.0**	0.2**	10/10**(83**)	10/10**(26**)	0.4**	0.0**
	0.25	5/5	3.2	10/10**	>21.0**	0.0**	10/10**(76**)	10/10**(20**)	0.0**	0.0**
	0.125	5/5	2.6	10/10**	>21.0**	0.6**	9/10**(1219**)	9/10**(1265**)	0.6**	0.7**
	0.0625	5/5	3.4	10/10**	>21.0**	0.1**	10/10**(80**)	10/10**(13**)	0.0**	0.0**
	0.031	5/5	2.8	10/10**	>21.0**	0.1**	9/10**(112**)	9/10**(52**)	0.5**	1.0**
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.2**	10/10**(76**)	10/10**(16**)	2.5**	4.1**
Saline	-	-	-	1/20	5.5	1.9	0/18(7919)	0/18(9653)	5.1	6.0
Normals	-	5/5	4.3	-	-	0.0	5/5(63)	5/5(9)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This experiment with poly IC•LC confirms the activity previously shown by us in PTA 307 (Table 15). The material was considered highly active vs PTV using all evaluation parameters.

*P<0.05 **P<0.01

Table V-37. Expt. PtA325. Effect of Once Daily Per Os Treatment with AVS1761 on Punta Toro Virus Infections in Mice.

Animals 12.5-13.7 g (3 wk) C57BL/6 Mice
Virus Adames strain Punta Toro virus, s.c. injected.
Drug Diluent H_2O.
Treatment Schedule: Once daily x 8, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected-Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1761	1	5/5	1.2	1/10	5.0	0.4**	1/8(4208)	2/8*(5313)	4.7	5.9
	0.5	5/5	2.3	1/10	5.8	2.3	0/9(9322)	0/9(11,456)	5.6	6.3
	0.25	5/5	4.0	0/10	5.0	1.2	0/9(6834)	0/9(9050)	5.7	6.3
	0.125	5/5	2.3	0/10	5.4	2.0	0/10(7100)	0/10(8355)	6.0	6.1
	0.0625	5/5	2.1	1/10	5.3	1.0	0/10(5422)	0/10(6101)	5.4	5.9
	0.031	5/5	2.9	0/10	5.4	1.8	0/10(5223)	1/10(5735)	5.3	6.2
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.2**	10/10**(78**)	10/10**(16**)	2.5**	4.1**
H_2O	-	-	-	1/20	5.2	1.7	1/19(7457)	0/19(7787)	5.2	6.1
Normals	-	5/5	4.3	-	-	0.0	5/5(63)	5/5(9)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean

Conclusions This experiment using poly IC•LC indicates, as found by others using other viruses, that the material is essentially ineffective when administered orally.

*P<0.05 **P<0.01

Table V-38. Expts. PtA326-331. Effect of Single i.p. Treatments with AVS1761 at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.0-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1761	4 hr pre	2.5	5/5	-0.7	10/10**	>21.0**
		1.25	5/5	-0.6	10/10**	>21.0**
		0.625	5/5	-0.5	10/10**	>21.0**
		0.31	5/5	-0.4	9/10**	5.0
	4 hr post	2.5	5/5	-0.7	10/10**	>21.0**
		1.25	5/5	-0.6	10/10**	>21.0**
		0.625	5/5	-0.5	10/10**	>21.0**
		0.31	5/5	-0.4	5/10**	3.8
	24 hr post	2.5	5/5	-0.7	10/10**	>21.0**
		1.25	5/5	-0.6	10/10**	>21.0**
		0.625	5/5	-0.5	10/10**	>21.0**
		0.31	5/5	-0.4	10/10**	>21.0**
	48 hr post	2.5	5/5	-0.7	4/10**	4.0
		1.25	5/5	-0.6	3/10*	4.9
		0.625	5/5	-0.5	5/10**	4.2
		0.31	5/5	-0.4	1/10	4.0
	72 hr post	2.5	5/5	-0.7	0/10	4.1
		1.25	5/5	-0.6	0/10	3.9
		0.625	5/5	-0.5	0/10	3.9
		0.31	5/5	-0.4	0/10	3.9
	96 hr post	2.5	5/5	-0.7	0/10	4.3
		1.25	5/5	-0.6	0/10	4.1
		0.625	5/5	-0.5	0/10	4.5
		0.31	5/5	-0.4	0/10	4.2
Ribavirin	350	5/5	0.3	7/10**	6.7**	
Saline	-	-	-	0/20	4.1	
Normals	-	5/5	0.3	-	-	

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Single i.p. treatment with poly IC•LC was effective against PTV when given as late as 48 hr after virus exposure

Table V-39. Expt. PtA243-248. Effect of Time of Single Treatment with AVS1767 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1767	4 hr pre	400	5/5	0.3	5/10*	5.8
		200	5/5	0.2	6/10**	5.5
		100	5/5	0.5	7/10**	5.3
		50	5/5	0.8	7/10**	5.3
		25	5/5	1.0	1/10	4.6
	4 hr post	400	5/5	0.3	7/10**	5.3
		200	5/5	0.2	6/10**	7.5
		100	5/5	0.5	4/10	5.5
		50	5/5	0.8	4/10	5.7
		25	5/5	1.0	5/10*	5.8
	24 hr post	400	5/5	0.3	7/10**	6.0
		200	5/5	0.2	6/10**	5.0
		100	5/5	0.5	4/10	5.8
		50	5/5	0.8	3/10	5.6
		25	5/5	1.0	4/9*	4.0
	48 hr post	400	5/5	0.3	7/10**	5.3
		200	5/5	0.2	1/10	5.1
		100	5/5	0.5	6/10**	5.8
		50	5/5	0.8	4/10	6.0
		25	5/5	1.0	7/10**	6.7
	72 hr post	400	5/5	0.3	2/10	4.8
		200	5/5	0.2	0/10	5.2
		100	5/5	0.5	4/10	6.2
		50	5/5	0.8	3/10	5.6
		25	5/5	1.0	1/10	5.7
	96 hr post	400	5/5	0.3	3/10	5.6
		200	5/5	0.2	1/10	5.9
		100	5/5	0.5	2/10	4.1
		50	5/5	0.8	2/10	5.1
		25	5/5	1.0	1/10	4.8
Ribavirin		350	5/5	0.2	10/10**	>21.0**
Saline		-	-	-	2/20	5.3
Normals		-	5/5	0.5	-	-

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Single treatments with AVS1767 (AM-3) were effective as late as 48 hr post-virus inoculation vs PTV *in vivo*. It is interesting and not surprising in view of the immunomodulatory effects of AM-3 that the most effective doses were often quite scattered.

Table V-40. Expt. PtA259. Effect of AVS1767 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1767	250	5/5	2.4	8/10	5.5
	125	5/5	2.7	10/10*	>21.0**
	62.5	5/5	2.5	10/10*	>21.0**
	31.3	4/5	2.7	3/10	6.7
Ribavirin	75	5/5	1.3	10/10**	>21.0**
CMC	-	-	-	12/20	5.6
Normals	-	5/5	3.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This experiment indicates that once daily s.c. treatment with AVS1767 (AM-3) is effective vs PTV *in vivo*; the activity is similar to that seen using this material twice daily (PtA 111).

Table V-41. Expt. PtA260-265. Effect of Time of Single Treatment with AVS1767 on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.4 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.

Treatment Schedule: Once only at varying times relative to virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days.

		Tox. Control			Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1767	4 hr pre	1000	5/5	-0.3	9/10**	7.0
		500	5/5	0.1	10/10**	>21.0**
		250	5/5	0.5	6/10	6.5
		125	5/5	0.5	6/10	6.3
		62.5	5/5	0.5	5/10	6.4
		31.3	5/5	0.8	5/10	5.8
		15.6	5/5	0.7	8/10**	4.5
	4 hr post	1000	5/5	-0.3	10/10**	>21.0**
		500	5/5	0.1	5/10	5.8
		250	5/5	0.5	2/10	5.5
		125	5/5	0.5	2/10	5.8
		62.5	5/5	0.5	8/10**	6.5
		31.3	5/5	0.8	1/10	5.7
		15.6	5/5	0.7	2/10	5.3
	24 hr post	1000	5/5	-0.3	2/10	5.9
		500	5/5	0.1	8/10**	7.5
		250	5/5	0.5	7/10*	5.3
		125	5/5	0.5	5/10	6.0
		62.5	5/5	0.5	7/10*	6.7
		31.3	5/5	0.8	2/10	6.1
		15.6	5/5	0.7	0/10	5.0
	48 hr post	1000	5/5	-0.3	7/10*	6.7
		500	5/5	0.1	3/10	5.3
		250	5/5	0.5	8/10**	4.5
		125	5/5	0.5	0/10	5.2
		62.5	5/5	0.5	10/10**	>21.0**
		31.3	5/5	0.8	8/10**	7.0
		15.6	5/5	0.7	8/10**	6.5
	72 hr post	1000	5/5	-0.3	2/10	5.3
		500	5/5	0.1	9/10**	5.0
		250	5/5	0.5	1/10	5.2
		125	5/5	0.5	2/10	5.1
		62.5	5/5	0.5	2/10	4.9
		31.3	5/5	0.8	0/10	5.7
		15.6	5/5	0.7	0/10	5.1
	96 hr post	1000	5/5	-0.3	2/10	4.8
		500	5/5	0.1	1/10	5.7
		250	5/5	0.5	3/10	4.7
		125	5/5	0.5	2/10	5.9
		62.5	5/5	0.5	6/10	5.0
		31.3	5/5	0.8	0/10	6.7
		15.6	5/5	0.7	4/10	7.6*
Ribavirin		350	5/5	0.3	8/10**	8.0
Saline		-	-	-	6/20	5.6
Normals		-	5/5	0.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This study indicates that AVS1767 (AM-3) can be administered as late as 72 hr post-virus inoculation and still be inhibitory to PTV. This is the first compound we have tested to exhibit activity when administered this late in the infection. Note the active dose levels often varied considerably, an expected observation with an immunomodulator

Table V-42. Expt. PtA308. Effect of Twice Daily i.p. Treatment With AVS1767 on Punta Toro Virus Infections in Mice.

Animals: 12.7-14.4 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1767	250	5/5	1.2	10/10**	>21.0**
	125	5/5	2.0	8/10**	6.0
	62.5	5/5	1.7	8/10**	7.0**
	31.3	5/5	2.4	7/10**	7.7**
	15.7	5/5	2.1	2/10	6.6**
Ribavirin	75	5/5	2.5	10/10**	>21.0**
Saline	-	-	-	2/20	4.9
Normals	-	5/5	3.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Twice daily i.p. treatment with AM-3 beginning 4 hr pre-virus inoculation were highly effective against PTV in this study.

Table V-43. Expt. PtA198-204. Effect of Time of Single Treatment of AVS1778 on Punta Toro Virus Infections in Mice.

Animals: 9.3-11.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: Once only, given at varying times relative to virus inoculation.
Treatment Route: s.c.

Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1778	24 hr pre	50	5/5	0.4	0/10	5.2
		25	5/5	-0.1	0/10	5.2
		12.5	5/5	0.2	0/10	5.1
		6.3	5/5	0.5	0/10	5.6
	4 hr pre	50	5/5	0.4	0/10	4.4
		25	5/5	-0.1	2/10	5.4
		12.5	5/5	0.2	1/10	5.3
		6.3	5/5	0.5	0/10	5.0
	4 hr post	50	5/5	0.4	0/10	4.0
		25	5/5	-0.1	0/10	4.3
		12.5	5/5	0.2	0/10	4.0
		6.3	5/5	0.5	0/10	4.3
	24 hr post	50	5/5	0.4	0/10	5.2
		25	5/5	-0.1	3/10	5.9*
		12.5	5/5	0.2	0/10	4.9
		6.3	5/5	0.5	1/10	4.7
	48 hr post	50	5/5	0.4	0/10	4.6
		25	5/5	-0.1	0/10	4.3
		12.5	5/5	0.2	0/10	4.5
		6.3	5/5	0.5	0/10	4.2
	72 hr post	50	5/5	0.4	1/10	4.3
		25	5/5	-0.1	0/10	4.2
		12.5	5/5	0.2	0/10	4.3
		6.3	5/5	0.5	1/10	4.0
	96 hr post	50	5/5	0.4	0/10	4.2
		25	5/5	-0.1	0/10	4.9
		12.5	5/5	0.2	0/10	4.1
		6.3	5/5	0.5	0/10	4.3
Ribavirin		75	5/5	2.6	10/10**	>21.0**
CMC		-	-	-	0/20	5.3
Normals		-	5/5	0.4	-	-

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is mannozym, shown previously to be active vs PTV in vivo when used s.c. on a bid x 5 or single treatment regimen. The present experiments were run to determine optimum times for single treatment. In the previous single treatment study, mannozym was given 4 hr pre-virus inoculation. Slight activity was seen when the material was administered 24 hr post-virus inoculation only, however. We cannot explain this failure to confirm our previous finding, seen in PtA 74.

Table V-44. Expt. PtA216. Effect of Once Daily Treatment with AVS1778 on Punta Toro Virus Infections in Mice.

Animals: 12.2-13.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1778	100	5/5	1.2	10/10**	>21.0**
	50	5/5	2.3	2/10	5.0**
	25	5/5	2.6	3/10	5.9**
	12.5	5/5	2.2	1/10	5.0**
	6.25	5/5	3.5	2/10	4.8*
	3.13	5/5	2.3	0/10	4.9*
Ribavirin	75	5/5	2.5	10/10**	>21.0**
CMC	-	-	-	2/20	4.2
Normals	-	5/5	2.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is mannozym, which previously was found effective vs PTV *in vivo* when administered s.c. twice daily for 5 days beginning 4 hr pre-virus inoculation. In that study (PtA 118), the material was shown to have a TI of 32. In this experiment, treatment once daily was less efficacious, with significant activity seen only at the highest dosage used.

Table V-45. Expt. PtA217. Effect of Twice Daily i.p. Treatment with AVS1778 on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1778	400	2/4	-1.5	-	-
	200	2/4	0.0	-	-
	100	4/5	1.2	0/9	4.4
	50	5/5	1.3	0/10	4.5
	25	5/5	0.6	0/10	4.6
	12.5	3/5	1.3	1/10	5.3
	6.25	4/5	-0.1	0/10	4.6
	3.13	4/5	-0.1	0/9	4.9
	1.56	3/5	0.9	0/9	4.3
	0.78	4/4	1.8	0/10	4.3
Ribavirin	75	5/5	2.5	10/10**	>21.0**
CMC	-	-	-	0/20	4.6
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound is mannozym, which has previously exhibited strong activity in two experiments vs PTV *in vivo* when administered s.c. by this treatment schedule (PTA 75, 118). The present study was run to determine if the material would also be effective when administered i.p. Surprisingly, nc activity was seen. Higher dosages were used for toxicity controls only to determine the MTD of this material. The MTD was considered to be 50 mg/kg/day.

Table V-46. Expt. PtA250. Effect of a Single Treatment with AVS1778 on Punta Toro Virus Infections in Mice.

Animals: 10.4-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1778	100	5/5	-1.0	8/10	8.5**
	50	5/5	-0.4	10/10**	>21.0**
	25	5/5	-0.2	3/10	6.0*
	12.5	5/5	-0.4	4/10	5.8
	6.25	5/5	0.3	8/10	4.5
Ribavirin	350	5/5	0.2	10/10**	>21.0**
CMC	-	-	-	7/20	4.9
Normals	-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Initially, AVS1778 (mannozym) was relatively effective vs PTV *in vivo* when administered in a single s.c. injection (PtA 74). Since our initial observations, a later lot of this material sent to us appeared less active, although used by other treatment regimens. We repeated the PtA 74 experiment, although using CMC vehicle instead of saline to determine if that initial activity was reproducible. Efficacy was seen primarily at the 50 mg/kg/day dose, with a weak effect also at 25 mg/kg/day, compared to activity at both 50 and 25 mg/kg/day doses seen in PtA 74. We do not consider the data from these two experiments to be significantly different.

Table V-47. Expt. PtA293. Effect of Once Daily Treatment with AVS1778 on Punta Toro Virus Infections in Mice.

Animals: 13.9-14.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1778	150	5/5	1.9	4/10	5.7
	75	5/5	2.0	6/10	7.0
	37.5	5/5	2.3	10/10**	>21.0**
	18.8	5/5	3.3	7/10*	6.0
	9.4	5/5	3.4	9/10**	10.0
Ribavirin	75	5/5	2.9	10/10**	>21.0**
CMC	-	-	-	6/20	6.0
Normals	-	5/5	3.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this study, AVS1778 (mannozym) was effective against PTV infections when administered once daily for 5 days via the s.c. route. These data compare well with earlier data using this compound on a twice daily s.c. treatment regimen (PtA 75, 118).

Table V-48. Expt. PtA294-296. Effect of Time of Treatment with AVS1778 on Punta Toro Virus Infections in Mice.

Animals: 13.9-15.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, at varying times relative to virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1778	4 hr pre	50	5/5	3.0	9/10**	10.0
		25	5/5	2.9	10/10**	>21.0**
		12.5	5/5	2.7	10/10**	>21.0**
		6.3	5/5	3.2	10/10**	>21.0**
		3.2	5/5	3.8	10/10**	>21.0**
		1.6	5/5	3.7	7/10*	6.0
	24 hr post	50	5/5	3.0	10/10**	>21.0**
		25	5/5	2.9	10/10**	>21.0**
		12.5	5/5	2.7	9/10**	5.0
		6.3	5/5	3.2	9/10**	4.0
		3.2	5/5	3.8	8/10**	11.0**
		1.6	5/5	3.7	10/10**	>21.0**
	48 hr post	50	5/5	3.0	6/10	11.3**
		25	5/5	2.9	7/10*	8.7
		12.5	5/5	2.7	7/10*	10.7**
		6.3	5/5	3.2	7/10*	5.7
		3.2	5/5	3.8	9/10**	5.0
		1.6	5/5	3.7	5/10	5.6
Ribavirin		75	5/5	2.9	10/10**	>21.0**
CMC		-	-	-	6/20	6.0
Normals		-	5/5	3.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: These data indicate that s.c. therapy with AVS1778 (mannozym) administered on a twice daily schedule can be initiated as late as 48 hr after PTV inoculation with marked efficacy seen.

Table V-49. Expt. PtA356. Effect of Single Per Os Treatment with AVS1969 24 hr Pre-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1969	200	5/5	-0.1	0/10	6.1	3.5	0/10(7305)	0/10(6020)	5.0	5.8
	20	5/5	0.7	0/10	4.9	1.9**	1/10(5055)	1/10(6026)	5.5	6.4
	2	5/5	0.1	0/10	5.0	2.2**	1/10(3483*)	1/10(4429)	4.9	6.1
Ribavirin	350	5/5	-0.4	10/10**	>21.0**	0.2**	10/10**(65**)	10/10**(30**)	3.4**	3.9**
H_2O	-	-	-	3/20	5.9	2.8	1/19(6824)	0/19(6391)	5.3	6.2
Normals	-	5/5	0.2	-	-	0.0	5/5(112)	5/5(28)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: This is CL259,763, an immune modulating substance described, in a recent meeting by Dr. Fred Durr of Lederle, as having a wide range of immunologic activities when administered p.o. in a single treatment. In the present series of experiments (PTA 356-360), however, the anti-PTV activity was considered very weak regardless of the time of treatment.

*P<0.05 **P<0.01

Table V-50. Expt. PtA357. Effect of Single Per Os Treatment with AVS1969 4 hr Pre-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.

Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1969	200	5/5	-0.1	1/10	4.7	1.2**	1/10(4117)	1/10(4654)	5.6	6.3
	20	5/5	0.7	0/10	5.4	2.2**	0/10(6978)	0/10(6160)	5.7	6.0
	2	5/5	0.1	1/10	5.9	2.2**	2/10(2050**)	1/10(4205)	5.4	6.2
Ribavirin	350	5/5	-0.4	10/10**	>21.0**	0.2**	10/10**(65**)	10/10**(30**)	3.4**	3.9**
H_2O	-	-	-	3/20	5.9	2.8	1/19(6824)	0/19(6391)	5.3	6.2
Normals	-	5/5	0.2	-	-	0.0	5/5(112)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: See the conclusions under PtA 356 (Table 20).

*P<0.05 **P<0.01

Table V-51. Expt. PtA358. Effect of Single Per Os Treatment with AVS1969 24 hr Post-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1969	200	5/5	-0.1	0/10	4.7	1.9**	0/10(4394)	0/10(7450)	5.3	6.4
	20	5/5	0.7	0/10	5.1	1.8**	0/10(6123)	0/10(7055)	5.4	6.3
	2	5/5	0.1	0/10	5.3	1.1**	0/9(3836)	0/9(4719)	4.9	6.1
Ribavirin	350	5/5	-0.4	10/10**	>21.0**	0.2**	10/10**(65**)	10/10**(30**)	3.4**	3.9**
H_2O	-	-	-	3/20	5.9	2.8	1/19(6824)	0/19(6391)	5.3	6.2
Normals	-	5/5	0.2	-	-	0.0	5/5(112)	5/5(28)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: See the conclusions under PtA 356 (Table 20).

*P<0.05 **P<0.01

Table V-52. Expt. PtA359. Effect of Single Per Os Treatment with AVS1969 48 hr Post-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 48 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1969	200	5/5	-0.1	0/10	4.6	1.6**	0/10(1722**)	0/10(4715)	5.4	6.0
	20	5/5	0.7	0/10	5.7	1.7**	2/9(1651**)	2/9(3769*)	5.0	5.3
	2	5/5	0.1	0/10	4.4	1.5**	4/9**(1559**)	2/9(3?9**)	4.9	5.9
Ribavirin	350	5/5	-0.4	10/10**	>21.0**	0.2**	10/10**(65**)	10/10**(30**)	3.4**	3.9**
H_2O	-	-	-	3/20	5.9	2.8	1/19(6824)	0/19(6391)	5.3	6.2
Normals	-	5/5	0.2	-	-	0.0	5/5(112)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: See the conclusions under PtA 356 (Table 20).

*P<0.05 **P<0.01

Table V-53. Expt. PtA360. Effect of Single Per Os Treatment with AVS1969 72 hr Post-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.6 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 72 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1969	200	5/5	-0.1	0/10	5.8	1.8**	1/10(2982**)	0/10(3903)	5.0	5.9
	20	5/5	0.7	2/10	4.6	1.4**	0/9(3294*)	0/9(3092*)	4.5**	5.8
	2	5/5	0.1	0/10	4.8	2.7	0/10(4444)	0/10(4475)	5.0	6.3
Ribavirin	350	5/5	-0.4	10/10**	>21.0**	0.2**	10/10**(65**)	10/10**(30**)	3.4**	3.9**
H_2O	-	-	-	3/20	5.9	2.8	1/19(6824)	0/19(6391)	5.3	6.2
Normals	-	5/5	0.2	-	-	0.0	5/5(112)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: See the conclusions under PtA 356 (Table 20).

*P<0.05 **P<0.01

Table V-54. Expt. PtA391-394. Effect of Once Only i.p. Treatment With AVS1969 Administered at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.6-15.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1969	24 hr pre	200	5/5	0.4	0/10	7.2*
		20	5/5	0.0	9/10**	6.0
		2	5/5	0.4	5/10	6.6
	4 hr pre	200	5/5	0.4	3/10	5.7
		20	5/5	0.0	0/10	4.8
		2	5/5	0.4	2/10	5.9
	24 hr pre	200	5/5	0.4	0/10	7.2*
		20	5/5	0.0	9/10**	6.0
		2	5/5	0.4	5/10	6.6
	24 hr post	200	5/5	0.4	0/10	4.9
		20	5/5	0.0	0/10	5.6
		2	5/5	0.4	2/10	6.0
	48 hr post	200	5/5	0.4	0/10	5.2
		20	5/5	0.0	0/10	4.7
		2	5/5	0.4	0/10	5.8
Ribavirin		350	5/5	-0.2	10/10**	>21.0**
CMC		-	-	-	8/20	5.3
Normals		-	5/5	0.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is the Lederle Immunomodulator CL259,763. Single treatment 24 hr pre-virus inoculation only was effective in this study.

Table V-55. Expt. PtA395. Effect of Twice Daily i.p. Treatment With AVS1969 on Punta Toro Virus Infections in Mice.

Animals: 11.5-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1969	100	5/5	2.6	0/10	4.1
	50	5/5	2.0	0/10	4.2
	25	5/5	2.6	2/10*	5.3**
	12.5	5/5	2.1	0/10	4.2
	6.25	5/5	2.7	0/10	4.5
Ribavirin	75	5/5	-	10/10**	>21.0**
CMC	-	-	-	0/20	4.2
Normals	-	5/5	3.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this study with the Lederle immunomodulator CL259,763, twice daily i.p. treatments were considered essentially ineffective.

Table V-56. Expt. PtA425. Effect of Twice Daily p.o. Treatment with AVS1969 on Punta Toro Virus Infections in Mice.

Animals: 13.3-14.9g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1969	200	5/5	1.3	0/10	3.9	3.6	0/9(17,844)	0/9(11,100)	5.8	6.3
	20	5/5	2.4	0/10	4.7	3.8	0/10(15,512)	0/10(10,020)	5.5	5.6
	2	5/5	1.7	0/10	4.2	3.7	0/10(16,123)	0/10(10,440)	5.8	5.9
Ribavirin[g]	75	5/5	2.6	10/10**	>21.0**	0.3**	10/10**(124**)	5/10**(120**)	1.2**	0.2**
CMC	-	-	-	5/20	5.1	3.8	0/19(17,366)	0/19(10,295)	5.5	6.1
Normals	-	5/5	3.8	-	-	0.4	4/5(230)	5/5(49)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]In sterile saline.

Conclusions: CL259763, an immunomodulator, was slightly active vs PTV in a previous series of experiments (PtA 356-360) when administered p.o. in a single treatment. Multiple p.o. treatment given in this experiment was totally ineffective.

*P<0.05 **P<0.01

Table V-57. Expt. PtA446. Effect of Twice Daily i.p. Treatment With AVS1976 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.5-13.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily x 5 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1976	100	5/5	2.1	0/10	4.0
	50	5/5	3.1	0/10	4.2
	25	5/5	3.2	0/10	4.6
	12.5	5/5	3.9	0/10	4.0
	6.25	5/5	2.9	0/10	4.2
Ribavirin	75	5/5	2.9	9/10**	11.0
Saline	-	-	-	0/20	4.3
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this initial test, thymine riboside 2',3'-dialdehyde was ineffective vs PTV. The material was reasonably well tolerated, suggesting higher dosages should be considered.

Table V-58. Expt. PtA452-454. Effect of Once Only i.p. Treatment With AVS1976 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.5-14.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1976	24 hr pre	500	0/5	-2.5	0/10	1.5
		250	5/5	-0.7	0/10	5.2**
		125	5/5	0.3	1/10	5.0**
		62.5	5/5	-0.2	0/10	5.4**
	4 hr pre	500	0/5	-2.5	0/10	2.3
		250	5/5	-0.7	3/10	4.7
		125	5/5	0.3	0/10	4.7
		62.5	5/5	-0.2	0/10	5.0**
	24 hr post	500	0/5	-2.5	0/10	2.9
		250	5/5	-0.7	0/10	4.3
		125	5/5	0.3	0/10	4.5
		62.5	5/5	-0.2	0/10	4.2
Ribavirin		350	5/5	0.3	10/10**	>21.0**
Saline		-	-	-	1/20	4.4
Normals		-	5/5	0.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS1976 (thymine riboside 2',3'-dialdehyde) was inactive in an initial test using a twice daily for 5 days i.p. treatment regimen (PtA 446). In the present study, single i.p. treatment was moderately efficacious using a single treatment.

Table V-59. Expt. PtA481. Effect of Twice Daily i.p. Treatment With AVS1976 on Punta Toro Virus Infections in Mice.

Animals: 12.8-14.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS1976	400	0/5	-	0/10	2.4
	200	5/5	-0.8	0/10	3.8
	100	5/5	1.3	0/10	4.3
	50	5/5	2.1	0/10	4.0
Ribavirin	75	5/5	1.5	9/10**	17.0
Saline	-	-	-	0/20	4.3
Normals	-	5/5	2.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In a previous experiment run with AVS1976 (thymine riboside 2',3'-dialdehyde) using this treatment regimen, no anti-PTV activity was seen, but the compound was nontoxic, so higher doses were run. Although toxicity was achieved, the compound was inactive in this experiment.

Table V-60. Expt. PtA205. Effect of Twice Daily Treatment with AVS2149 Beginning 4 hr Pre-virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 10.0-10.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2149	5	5/5	1.6	10/10**	>21.0**
	2.5	5/5	2.5	10/10**	>21.0**
	1.25	5/5	2.2	10/10**	>21.0**
	0.625	5/5	2.2	10/10**	>21.0**
	0.31	5/5	2.3	8/10**	7.5
Ribavirin	75	5/5	2.8	9/10**	11.0
Saline	-	-	-	0/20	5.2
Normals	-	5/5	1.3[c]	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Weight two days earlier than others

*P<0.05 **P<0.01

Conclusions: This compound is ampligen, which previously was shown to be effective vs PTV *in vivo* when administered once only, once daily for up to 8 days, or every other day for 4 days. This experiment was run to determine the effects of a twice daily x 5 treatment schedule. Marked anti-PTV effects were seen.

Table V-61. Expt. PtA207. Effect of Once Daily i.p. Treatment with AVS2149 on Punta Toro Virus Infections in Mice.

Animals: 12.8-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily X 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2149	25	3/3	1.7	10/10**	>21.0**
	12.5	5/5	1.6	10/10**	>21.0**
	6.25	5/5	2.3	10/10**	>21.0**
	3.13	5/5	1.9	10/10**	>21.0**
Ribavirin	75	5/5	2.5	10/10**	>21.0**
Saline	-	-	-	6/20	5.2
Normals	-	5/5	3.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This study was run to further elucidate the best treatment regimen for ampligen. In this study, an i.p. once daily x 5 treatment schedule was highly efficacious; compare with PtA 142, where the compound was given by the same schedule but via oral gavage route and was only weakly effective.

Table V-62. Expt. PtA208-214. Effect of Time of Single Treatment with AVS2149 on Punta Toro Virus Infections in Mice.

Animals: 12.2-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2149	24 hr pre	10	5/5	0.0	9/10**	8.0
		5	5/5	0.3	9/10**	9.0
		2.5	5/5	0.4	9/10**	7.0
		1.25	5/5	0.1	10/10**	>21.0**
	4 hr pre	10	5/5	0.0	10/10**	>21.0**
		5	5/5	0.3	10/10**	>21.0**
		2.5	5/5	0.4	10/10**	>21.0**
		1.25	5/5	0.1	9/10**	7.0
	4 hr post	10	5/5	0.0	10/10**	>21.0**
		5	5/5	0.3	9/10**	11.0
		2.5	5/5	0.4	10/10**	>21.0**
		1.25	5/5	0.1	7/10**	9.7**
	24 hr post	10	5/5	0.0	10/10**	>21.0**
		5	5/5	0.3	10/10**	>21.0**
		2.5	5/5	0.4	10/10**	>21.0**
		1.25	5/5	0.1	10/10**	>21.0**
	48 hr post	10	5/5	0.0	10/10**	>21.0**
		5	5/5	0.3	10/10**	>21.0**
		2.5	5/5	0.4	10/10**	>21.0**
		1.25	5/5	0.1	3/10*	5.7*
	72 hr post	10	5/5	0.0	0/10	4.1
		5	5/5	0.3	0/10	4.0
		2.5	5/5	0.4	0/10	4.1
		1.25	5/5	0.1	0/10	4.3
	96 hr post	10	5/5	0.0	0/10	4.3
		5	5/5	0.3	0/10	4.1
		2.5	5/5	0.4	0/10	4.2
		1.25	5/5	0.1	0/10	4.3
Ribavirin		200	5/5	0.2	3/10*	5.4
Saline		-	-	-	0/20	4.9
Normals		-	5/5	1.2	-	-

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This study indicates that AVS2149 (ampligen) can be administered i.p. in single injections as late as 48 hr post-virus inoculation and still be markedly inhibitory to PTV *in vivo*. Treatments begun 72 or 96 hr post-virus inoculation were ineffective.

Table V-63. Expt. PtA215. Effect of Twice Daily Treatment with AVS2149 Beginning 24 hr Post-virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.9-12.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2149	5	5/5	1.5	10/10**	>21.0**
	2.5	5/5	1.8	10/10**	>21.0**
	1.25	5/5	1.6	10/10**	>21.0**
	0.6	5/5	2.6	8/8**	>21.0**
Ribavirin	75	5/5	1.9	10/10**	>21.0**
Saline	-	-	-	1/20	5.4
Normals	-	5/5	2.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound is ampligen, which was previously found effective given twice daily for 5 days beginning prior to virus inoculation (PtA 205). In this experiment, the same treatment begun 24 hr after virus inoculation was similarly efficacious.

Table V-64. Expt. PtA257. Effect of Single Daily Treatments with AVS2149 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2149	5	5/5	1.8	10/10**	>21.0**
	2.5	5/5	1.1	10/10**	>21.0**
	1.25	5/5	1.1	10/10**	>21.0**
	0.625	5/5	2.3	10/10**	>21.0**
	0.31	5/5	1.6	10/10**	>21.0**
Ribavirin	38	5/5	2.1	10/10**	>21.0**
Saline	-	-	-	9/20	5.2
Normals	-	5/5	3.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Once daily i.p. treatment with AVS2149 (ampligen) beginning 4 hr pre-virus inoculation was markedly effective vs PTV *in vivo*. This activity compares well with treatment beginning 24 hr pre (PtA 56).

Table V-65. Expt. PtA309, 310. Effect of Late i.p. Therapy With AVS2149 on Punta Toro Virus Infections in Mice.

Animals: 12.7-14.4 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, 72 or 96 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control			Infected, Treated	
Compound	Treatment Initiation	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2149	72 hr post	5	5/5	0.9	0/10	4.0
		2.5	5/5	1.6	0/10	4.3
		1.25	5/5	1.5	0/10	4.5
		0.625	5/5	1.5	0/10	4.5
	96 hr post	5	5/5	0.9	0/10	4.3
		2.5	5/5	1.6	0/10	4.8
		1.25	5/5	1.5	1/10	5.0
		0.625	5/5	1.5	0/10	4.2
Ribavirin	24 hr post	75	5/5	2.5	10/10**	>21.0**
Saline		-	-	-	1/20	4.8
Normals		-	5/5	1.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: We have previously shown that qd x 5 i.p. treatments with AVS2149 (ampligen) beginning as late as 48 hr after PTV inoculation were highly active against the disease. This experiment utilized similar therapy beginning 72 or 96 hr after virus inoculation. No activity was seen if initiated this late in the infection, however.

Table V-66. Expts. PtA518-521. Effect of Once Only i.p. Treatment With AVS2149 on Varying Concentrations of Punta Toro Virus Infections in Mice.

Animals: 13.3-15.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected. At varyious conc.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Virus Dilution	Dosage (mg/kg)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2149	$10^{-2.0}$	5	5/5	-0.1	10/10**	>21.0**
		1.6	5/5	0.4	10/10**	>21.0**
		0.5	4/5	0.5	10/10**	>21.0**
		0.16	5/5	0.5	8/10**	7.0
		0.05	5/5	0.0	8/10**	5.5
		0.016	5/5	0.3	8/10**	4.5
Saline		-	-	-	3/20	5.1
AVS2149	$10^{-3.0}$	5	5/5	-0.1	10/10**	>21.0**
		1.6	5/5	0.4	10/10**	>21.0**
		0.5	4/5	0.5	10/10**	>21.0**
		0.16	5/5	0.5	10/10**	>21.0**
		0.05	5/5	0.0	8/10**	5.5
		0.016	5/5	0.3	9/10**	5.0
Saline		-	-	-	0/20	4.5
AVS2149	$10^{-4.0}$	5	5/5	-0.1	10/10**	>21.0**
		1.6	5/5	0.4	9/10**	6.0
		0.5	4/5	0.5	9/10**	6.0
		0.16	5/5	0.5	8/10**	8.0
		0.05	5/5	0.0	9/10**	6.0
		0.016	5/5	0.3	8/10**	6.0
Saline		-	-	-	0/20	4.5
AVS2149	$10^{-5.0}$	5	5/5	-0.1	10/10**	>21.0**
		1.6	5/5	0.4	10/10**	>21.0**
		0.5	4/5	0.5	8/10**	6.0
		0.16	5/5	0.5	10/10**	>21.0**
		0.05	5/5	0.0	7/10**	4.7
		0.016	5/5	0.3	3/10	4.7
Saline		-	-	-	3/20	5.2
Normals	-	-	5/5	0.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS2149 (ampligen) was highly active against all concentrations of challenge PTV inoculum

Table V-67. Expt. PtA432. Effect of Twice Daily i.p. Treatment With AVS2700 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 13.4-14.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2700	400	0/5^	-1.3	0/10	7.0**
	200	5/5	-0.2	3/10*	9.1**
	100	5/5	1.4	4/10**	7.2**
	50	5/5	1.6	10/10**	>21.0**
	25	5/5	1.5	10/10**	>21.0**
Ribavirin	75	5/5	1.9	10/10**	>21.0**
Saline	-	-	-	0/20	5.0
Normals	-	5/5	2.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

^Mean survival time of toxicity control mice at this dosage level was 11.0 days.

*P<0.05 **P<0.01

Conclusions: In this initial experiment, 6-ethyl thiopurine riboside was considered highly effective vs PTV. The highest dosage was toxic to the mice; the 200 mg/kg/day dose was also slightly toxic as indicated by host weight loss in the toxicity controls. This experiment will be repeated to confirm these data.

Table V-68. Expt. PtA450. Effect of Twice Daily i.p. Treatment with AVS2700 on Punta Toro Virus Infections in Mice.

Animals: 11.8-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

	Toxicity controls			Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2700	100	5/5	2.1	0/10	4.7	4.0	0/10(6404)	0/10(8445)	6.3	5.9
	50	5/5	2.4	2/10*	4.1	4.0	0/10(16,200)	0/10(12,500)	7.2	6.5
	25	5/5	3.4	0/10	4.1	3.6	0/8(13,869)	0/8(11,506)	6.4	6.3
	12.5	5/5	2.5	1/10	4.1	3.6	0/9(14,484)	0/9(11,144)	6.8	6.4
	6.25	5/5	3.3	0/10	4.4	4.0	0/10(16,200)	0/10(12,500)	7.2	6.5
	3.13	5/5	2.6	0/10	4.2	2.8	0/10(14,360)	0/10(11,345)	6.9	6.5
Ribavirin	75	5/5	2.3	10/10**	>21.0**	0.2**	8/10**(166**)	10/10**(39**)	0.3**	0.3**
Saline	-	-	-	0/20	4.2	3.7	0/20(8992)	0/20(9019)	5.0	5.5
Normals	-	5/5	3.0	-	-	0.1	5/5(116)	5/5(23)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: 6-Ethylthiopurine riboside was shown initially (PtA 432) to be highly active vs PTV in mice. This activity was not confirmed in the present study, however. WE note that 2 vials of AVS2700 were sent to us by Technassociates. The initial positive activity was seen using compound from the first vial. The second vial was used in this study, suggesting the same chemical may not have been in both vials. This experiment will be repeated again.

*P<0.05 **P<0.01

Table V-69. Expt. PtA473. Effect of Twice Daily i.p. Treatment with AVS2700 on Punta Toro Virus Infections in Mice.
Animals: 12.4-14.5 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2700	50	5/5	1.2	1/10	4.4	3.1	0/10(16,507)	0/10(10,703)	5.8	5.9
	25	5/5	2.2	0/10	5.0	3.5	0/7(15,529)	0/7(9779)	5.7	6.1
	12.5	4/4	2.9	0/10	4.4	3.5	0/9(21,128)	0/9(12,500)	6.2	6.4
	6.25	5/5	1.9	0/10	4.9	2.9	0/9(16,071)	0/9(10,705)	5.5	5.3
	3.125	5/5	2.4	0/10	3.4	3.9	0/10(19,050)	0/10(11,200)	6.3	6.5
	1.56	5/5	2.3	0/10	4.2	3.8	0/9(20,067)	0/9(11,878)	6.3	6.4
Ribavirin	75	5/5	1.4	9/10**	10.0	0.1**	9/10**(143**)	10/10**(60**)	0.4**	1.7**
Saline	-	-	-	2/20	4.7	3.4	0/17(15,723)	0/17(10,354)	5.7	5.9
Normals	-	5/5	2.4	-	-	0.0	4/5(246)	5/5(52)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: 6'-Ethylthiopurine riboside was again inactive vs PTV in this third experiment. Our only explanation is that the original test (PtA 432) used a different compound.

*P<0.05 **P<0.01

Table V-70. Expt. PtA305. Effect of Once Daily i.p. Treatment With AVS2712 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.2-14.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline + EtOH.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (µg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2712	36	5/5	2.7	0/10	4.5
	18	5/5	2.8	7/10**	5.3
	9	5/5	2.8	0/10	4.1
	4.5	5/5	2.9	1/10	4.3
Ribavirin	75[c]	5/5	2.6	10/10**	>21.0**
Saline + EtOH	-	-	-	1/20	4.7
Normals	-	5/5	1.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]mg/kg/day.

*P<0.05 **P<0.01

Conclusions: This is the initial test for bryostatin 1, with the dosages and treatment regimen as recommended by USAMRIID. Activity, expressed as 65% increase in survivors, was seen only at the next to the highest dose used. This is unusual, since the higher dose was apparently well tolerated, unless the compound has an immune modulating effect in the mouse. The experiment will be repeated to confirm the activity seen, if sufficient material is available.

Table V-71. Expt. PtA379. Effect of Twice Daily i.p. Treatment With AVS2712 on Punta Toro Virus Infections in Mice.

Animals: 12.7-13.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% EtOH + Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (ug/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2712	50	5/5	2.1	0/10	4.6
	25	5/5	2.0	1/10	4.2
	12.5	5/5	2.3	0/10	5.5*
	6.25	5/5	2.7	1/10	5.6
Ribavirin	75[c]	5/5	2.4	10/10**	>21.0**
Saline	-	-	-	1/20	4.4
Normals	-	5/5	3.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]mg/kg/day.

*P<0.05 **P<0.01

Conclusions: Bryostatin 1 was found active at a single dose when used qd x 5 i.p. (PtA 306, Table 26). A bid x 5 treatment schedule used in the present study was apparently ineffective

Table V-72. Expt. PtA426. Effect of Once Daily i.p. Treatment With AVS2712 on Punta Toro Virus Infections in Mice.

Animals: 13.4-14.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% EtOH in saline.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
	Dosage	Surv/	Host Wt.	Surv/	MST[b]
Compound	(µg/kg/day)	Total	Change (g)[a]	Total	(days)
AVS2712	18	5/5	2.8	4/10	4.5
	9	5/5	2.4	4/10	5.3
	4.5	5/5	3.5	0/10	5.6
	2.25	5/5	3.0	0/10	5.2
Ribavirin	75	5/5	2.4	10/10**	>21.0**
Saline	-	5/5	3.1	4/20	5.0
Normals	-	5/5	3.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Bryostatin I was moderately active in a previous experiment (PtA 305) using this treatment schedule, but the activity was seen only at a single dose (18 mg/kg/day). That positive result could not be confirmed in the present study.

Table V-73. Expt. PtA503. Effect of Once Daily i.p. Treatment with AVS2712 on Punta Toro Virus Infections in Mice.

Animals: 11.8-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% EtOH in Saline.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (µg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2712	144	5/5	1.7	3/10	4.4	3.6	0/10(6751)	0/10(6985)	5.1	5.8
	72	5/5	2.5	0/10	5.8**	3.9	0/10(7804)	0/10(8635)	5.2	6.1
	36	5/5	2.2	0/10	5.2	3.3	0/7(5497)	0/7(6188)	4.8	5.5
	18	5/5	1.7	4/10	4.8	2.4	1/8(2461)	0/8(2566)	4.5	4.8
	9	5/5	1.8	0/10	4.7	3.6	0/9(9838)	0/9(11,489)	5.7	6.4
	4.5	5/5	2.8	2/10	6.0*	3.4	0/7(8943)	0/7(10,050)	5.5	6.3
Ribavirin	75	5/5	2.5	10/10**	>21.0**	0.1**	7/10**(132**)	10/10**(30**)	2.4**	3.1**
Saline	-	-	-	2/20	4.7	2.7	0/19(2622)	0/19(2631)	4.6	5.1
Normals	-	5/5	2.7	-	-	0.1	3/5(153)	5/5(20)	0.6	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AVS2712 (bryostatin 1) was essentially inactive when given by this once daily treatment regimen.

*P<0.05 **P<0.01

Table V-74. Expt. PtA509-510. Effect of Once Only i.p. Treatment With AVS2712 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 13.2-13.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% EtOH.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (µg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2712	24 hr pre	200	5/5	0.0	5/10**	5.6
		100	5/5	0.2	0/10	6.0
		50	5/5	0.4	1/10	5.7
		25	5/5	0.4	0/10	5.6
		12.5	5/5	0.3	2/10*	6.3
		6.25	5/5	0.2	0/10	5.9
	4 hr post	200	5/5	0.0	4/10**	5.8
		100	5/5	0.2	0/10	6.8
		50	5/5	0.4	1/10	5.4
		25	5/5	0.4	0/10	5.5
		12.5	5/5	0.3	5/10**	6.4
		6.25	5/5	0.2	1/10	5.1
Ribavirin		350	5/5	0.0	10/10**	>21.0**
EtOH		-	-	-	0/20	5.5
Normals		-	5/5	0.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this experiment, AVS2712 (bryostatin 1), was significantly active vs PTV at two dosages when administered in a single i.p. injection. Previous work using multiple once or twice daily therapy was less effective.

Table V-75. Expt. PtA306. Effect of Once Daily i.p. Treatment With AVS2713 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.4-13.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline + EtOH.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (µg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2713	36	5/5	2.3	0/10	4.6
	18	5/5	3.4	0/10	4.8
	9	5/5	3.4	0/10	4.4
	4.5	5/5	3.5	0/10	4.9
Ribavirin	75[c]	5/5	2.6	10/10**	>21.0**
Saline + EtOH	-	-	-	1/20	4.7
Normals	-	5/5	1.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]mg/kg/day

*P<0.05 **P<0.01

Conclusions: In this initial test with bryostatin 2, with the dosages and treatment regimen as recommended by USAMRIID, no anti-PTV activity was seen. The material was well tolerated at all dosages, however, suggesting we may be using concentrations which are too low in order for efficacy to be seen.

Table V-76. Expt. PtA380. Effect of Twice Daily i.p. Treatment With AVS2713 on Punta Toro Virus Infections in Mice.

Animals: 12.3-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 2% EtOH + Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (µg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2713	40	5/5	2.8	1/10	5.0
	20	5/5	1.9	1/10	5.0
	10	5/5	3.4	0/10	4.6
	5	5/5	3.0	1/10	5.0
Ribavirin	75[c]	5/5	2.4	10/10**	>21.0**
Saline	-	-	-	1/20	4.4
Normals	-	5/5	3.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]mg/kg/day.

*P<0.05 **P<0.01

Conclusions: In this study with Bryostatin 2 using a bid x 5 treatment, no efficacy was seen. A previous test (PtA 306) used a qd x 5 schedule with also no anti-PTV activity seen. Again in this study, the material was well tolerated indicating higher dosages should be studied if compound was available.

Table V-77. Expt. PtA297. Effect of AVS2741 on Punta Toro Virus Infections in Mice.

Animals: 13.9-15.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2741	600	5/5	3.9	1/10	4.9
	300	5/5	3.6	4/10	6.2*
	150	5/5	3.8	0/10	5.9*
	75	5/5	3.9	1/10	5.1
Ribavirin	75	5/5	3.2	10/10*	>21.0**
Saline	-	-	-	12/20	4.6
Normals	-	5/5	3.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this experiment, AVS2741 (1-(ß-D-ribofuranosyl)-1,2,4-triazole-3-(1,4,5,6-tetrahydropyrimidine)•HCl) was slightly effective vs PTV infections, as seen by significant increases in mean survival time at two dosage levels. The material was well tolerated at the dosages used in this study.

Table V-78. Expt. PtA254. Effect of Per Os Once Daily Treatment with AVS2776 on Punta Toro Virus Infections in Mice.

Animals: 10.0-12.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Once daily x 3, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

	Toxicity controls			Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2776	400	5/5	-1.0	10/10**	>21.0**	0.8	9/9(54*)	9/9(12**)	2.2	0.0**
	200	5/5	2.0	10/10**	>21.0**	0.3**	10/10(80)	10/10(21**)	2.0	1.8
	100	5/5	1.3	10/10**	>21.0**	0.5	9/10(100)	10/10(24**)	2.4	0.8**
	50	5/5	1.9	7/10	6.3	0.4**	8/10(170)	7/10(158)	3.0	2.9
	25	5/5	2.2	8/10*	6.0	1.2	10/10(67*)	10/10(19**)	3.1	0.6**
Ribavirin	75	5/5	3.4	4/5	7.0	0.3**	4/4(76)	3/4(68)	2.9	3.8
CMC	-	-	-	7/20	6.0	1.7	15/20(652)	15/20(629)	2.5	2.8
Normals	-	5/5	2.5	-	-	0.3	5/5(43)	5/5(9)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: Earlier experiments showed that AVS2776 (ABPP) was effective vs PTV *in vivo* when administered i.p. The present experiment indicates ABPP to also be markedly effective when administered by oral gavage once daily for 3 days.

*P<0.05 **P<0.01

Table V-79. Expt. PtA255. Effect of Single Per Os Treatment with AVS2776 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Once only, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

	Toxicity controls			Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2776	400	5/5	1.2	10/10**	>21.0**	0.3	9/9(70**)	9/9(16**)	1.9**	2.0**
	200	5/5	1.1	10/10**	>21.0**	0.4	10/10(96**)	10/10(20**)	0.8**	1.0**
	100	5/5	1.0	9/10**	9.0	0.4	10/10(49**)	10/10(13**)	0.0**	0.0**
	50	5/5	1.3	5/10	5.6	0.6	5/8(600)	5/8(575)	3.5	2.3**
	25	5/5	-0.1	10/10**	>21.0**	0.7	0/10(1625)	0/10(1455)	5.4	6.3
Ribavirin	350	5/5	0.2	4/10	6.0	0.5	7/10(748)	7/10(274)	2.9	3.7
CMC	-	-	-	7/20	6.0	0.8	11/19(1147)	12/19(347)	4.0	4.8
Normals	-	5/5	2.5	-	-	0.3	5/5(43)	5/5(9)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Earlier experiments showed that AVS2776 (ABPP) was effective vs PTV *in vivo* when administered i.p. The present experiment indicates ABPP to also be markedly effective when administered by oral gavage in a single treatment.

*P<0.05 **P<0.01

Table V-80. Expt. PtA256. Effect of Single Subcutaneous Treatment with AVS2776 on Punta Toro Virus Infections in Mice.

Animals: 10.1-12.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2776	400	5/5	0.0	10/10**	>21.0**
	200	5/5	-0.1	10/10**	>21.0**
	100	5/5	0.3	7/10	6.7
	50	5/5	0.2	2/10	5.6
Ribavirin[c]	350	5/5	0.1	9/10*	7.0
CMC	-	-	-	9/20	6.0
Normals	-	5/5	0.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or befcre day 21.

[c]Ribavirin given 4 hr pre-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This experiment demonstrates that s.c. treatment with AVS2776 (ABPP) is also effective vs PTV *in vivo*.

Table V-81. Expt. PtA312. Effect of Late Once Daily Oral Treatment with AVS2776 on Punta Toro Virus Infections in Mice.

Animals: 13.5-14.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 3, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls	Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Surv/ Total	MST[a] (days)	Mean Liver Score[b]	SGOT Neg/Total[c] (Mean)	SGPT Neg/Total[d] (Mean)	Mean Liver Virus Titee (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2776	200	5/5	9/10**	4.0	0.4**	8/9**(106**)	8/9**(107**)	2.5**	4.1
	100	5/5	9/10**	7.0	0.8**	8/10**(691**)	5/10**(1342*)	3.4**	4.7
	50	5/5	2/10*	4.5	0.3**	4/10(705**)	4/10(749**)	3.0**	4.3
	25	5/5	2/10*	4.9	2.2	1/6(7443)	1/6(7862)	4.6	6.2
	12.5	5/5	0/10	4.6	0.8**	1/8(1953**)	1/8(1634**)	4.5	5.6
Ribavirin	100	5/5	10/10**	>21.0**	0.4**	7/9**(199**)	4/9(169**)	1.9**	3.9**
CMC	-	-	0/20	4.6	1.8	3/16(5055)	3/16(5094)	5.0	5.5
Normals	-	5/5	-	-	0.2	5/5(75)	5/5(10)	3.5	0.0

[a]Mean survival time of mice dying on or before day 21.

[b]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[c]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[d]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[e]Geometric mean.

Conclusions: In this study, ABPP was highly active vs PTV using all evaluation parameters when administered p.o beginning 24 hr after virus inoculation.

*P<0.05 **P<0.01

Table V-82. Expts. PtA413-416. Effect of Once Only p.o. Treatment With AVS2776 on Varying Concentrations of Punta Toro Virus Infections in Mice.

Animals: 12.6-13.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected. At varyious conc.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Virus Dilution	Dosage (mg/kg)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2776	$10^{-1.0}$	400	5/5	1.1	10/10*	>21.0**
		200	5/5	0.4	10/10*	>21.0**
		100	4/5	0.7	10/10*	>21.0**
		50	5/5	1.1	10/10*	>21.0**
		25	5/5	1.0	8/10	4.0
CMC		-	-	-	13/20	5.0
AVS2776	$10^{-2.0}$	400	5/5	1.1	10/10**	>21.0**
		200	5/5	0.4	10/10**	>21.0**
		100	4/5	0.7	10/10**	>21.0**
		50	5/5	1.1	8/10**	6.0
		25	5/5	1.0	3/10	4.7
CMC		-	-	-	7/20	5.0
AVS2776	$10^{-3.0}$	400	5/5	1.1	10/10**	>21.0**
		200	5/5	0.4	10/10**	>21.0**
		100	4/5	0.7	10/10**	>21.0**
		50	5/5	1.1	9/10**	
		25	5/5	1.0	7/10	6.3
CMC		-	-	-	9/20	5.2
AVS2776	$10^{-4.0}$	400	5/5	1.1	10/10**	>21.0**
		200	5/5	0.4	10/10**	>21.0**
		100	4/5	0.7	10/10**	>21.0**
		50	5/5	1.1	9/10*	6.0
		25	5/5	1.0	6/10	5.8
CMC		-	-	-	11/20	5.3
Normals	-	-	5/5	0.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this study, ABPP was evaluated to determine if increasing virus challenge would influence the efficacy of the compound. The positive anti-PTV data were essentially the same at every virus dose, however, the number of animals dying in placebo-treated controls did not correlate well with virus inoculum size. This study will be repeated.

Table V-83. Expts. PtA474-477. Effect of Once Only p.o. Treatment With AVS2776 on Varying Concentrations of Punta Toro Virus Infections in Mice.

Animals: 12.6-13.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected. At varyious conc.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Virus Dilution	Dosage (mg/kg)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2776	$10^{-1.0}$	400	5/5	0.1	10/10**	>21.0**
		200	5/5	0.8	9/10**	5.0
		100	4/5	0.5	8/10**	5.0
		50	5/5	-0.1	6/10	6.3**
		25	5/5	0.6	3/10	5.1
CMC		-	-	-	6/20	4.9
AVS2776	$10^{-2.0}$	400	5/5	0.1	10/10**	>21.0**
		200	5/5	0.8	10/10**	>21.0**
		100	4/5	0.5	8/10**	5.0
		50	5/5	-0.1	7/10**	5.7
		25	5/5	0.6	1/10	4.6
CMC		-	-	-	1/20	5.3
AVS2776	$10^{-3.0}$	400	5/5	0.1	10/10**	>21.0**
		200	5/5	0.8	10/10**	>21.0**
		100	4/5	0.5	10/10**	>21.0**
		50	5/5	-0.1	2/10	5.3
		25	5/5	0.6	1/10	4.4
CMC		-	-	-	3/20	4.6
AVS2776	$10^{-4.0}$	400	5/5	0.1	10/10**	>21.0**
		200	5/5	0.8	9/10**	5.0
		100	4/5	0.5	6/10*	7.3**
		50	5/5	-0.1	4/10	7.7**
		25	5/5	0.6	1/10	4.8
CMC		-	-	-	4/20	5.7
Normals	-	-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This study was run to repeat PtA413-416, in which ABPP was run vs several virus concentrations. The compound's efficacy seemed not to be influenced by increasing viral dosage.

Table V-84. Expt. PtA231. Effect of Oral Treatment with AVS2777 on Punta Toro Virus Infections in Mice.

Animals: 11.8-12.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Once daily X 3, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2777	400	5/5	1.3	7/10**	6.0	0.2**	10/10(243)	9/10(101)	1.1**	1.7*
	200	5/5	-0.5	1/10	6.0	0.8	10/10(112)	10/10(62*)	3.5	4.5
	100	5/5	0.5	1/10	6.3	0.9	8/10(566)	8/10(413)	5.4	6.4
	50	5/5	0.5	1/10	5.9	0.4**	10/10(57*)	9/9(11*)	1.5*	1.1**
CMC	-	-	-	0/10	6.9	0.8	10/10[g](183)	9/10[g](156)	3.8	4.0
Normals	-	5/5	1.8	-	-	0.0	5/5(59)	5/5(16)	0.7	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <900 Sigma-Frae.kel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]It is questionable whether the CMC controls were infected with the proper concentration of virus.

Conclusions: This is AIPP, an immunomodular which we have previously found to have significant activity vs PTV *in vivo* when administered i.p. The present study was run to determine if p.o. treatment would also be effective. Moderate activity was seen at the highest and lowest dosage used.

*P<0.05 **P<0.01

Table V-85. Expt. PtA313. Effect of Single Oral Treatment with AVS2777 on Punta Toro Virus Infections in Mice.

Animals: 13.5-14.7 g (3-wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2777	200	5/5	0.7	0/10	4.6	1.5	0/10(7665)	0/10(8980)	4.4	6.1
	100	5/5	0.8	1/10	5.0	1.7	0/10(5949)	0/10(7840)	5.2	6.3
	50	5/5	0.4	1/10	5.0	1.2	3/10*(2457**)	3/10*(2203**)	4.8	5.3
	25	5/5	1.0	0/10	5.0	1.2	3/10*(3419**)	4/10**(3188**)	3.9*	5.1
Ribavirin	350	5/5	0.2	5/10**	6.8**	1.0*	2/8*(1181**)	1/8(1312**)	4.5	5.7
CMC	-	-	-	1/20	4.7	1.8	0/18(6475)	0/18(6933)	5.1	5.9
Normals	-	5/5	0.5	-	-	0.1	5/5(60)	5/5(12)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: AIPP was essentially inactive vs PTV when administered orally prior to infection.

*P<0.05 **P<0.01

Table V-86. Expt. PtA235. Effect of a Single I.p. Treatment with AVS2778 on Punta Toro Virus Infections in Mice (Confirming Experiment).

Animals: 10.9-13.5 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2778	800	5/5	-0.9	9/10**	6.0	1.0	9/9(44**)	9/9*(12**)	0.6**	0.5**
	400	5/5	-0.5	8/10**	6.5	0.1**	10/10(48**)	10/10*(16**)	1.0**	0.6**
	200	5/5	-0.8	9/10**	8.0	0.6	9/9(154)	7/9(256)	3.2	3.7
	100	5/5	0.5	3/10	5.9	0.5*	10/10(126)	7/10(371)	2.3	2.6
	50	5/5	0.8	6/10	5.8	0.5*	9/9(51**)	9/9*(11**)	1.1*	0.7**
Ribavirin	350	5/5	0.4	8/10**	6.0	0.1**	10/10(154)	10/10*(65**)	2.6	3.2
CMC	-	-	-	6/20	6.8	0.9	15/20(423)	14/20(376)	2.7	2.8
Normals	-	4/5^	0.1	-	-	0.2	4/4(62)	4/4(38)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 5 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <900 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <600 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^One mouse found dead on Day 12, had too many teeth on bottom jaw.

Conclusions: In this experiment, single i.p. treatment with AVS2778 (ABMP) was found to be highly effective vs PTV *in vivo*, with efficacy seen using several infection parameters. This confirms and extends our previous initial observation (PtA 66).

*P<0.05 **P<0.01

Table V-87. Expt. PtA274. Effect of Single Per Os Treatment with AVS2778 on Punta Toro Virus Infections in Mice.

Animals: 10.9-13.5 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2778	400	5/5	0.6	6/10	6.8	0.6**	7/9(1922)	7/9(1614)	2.3**	2.4**
	200	5/5	-0.2	5/10	6.2	0.8	8/10(449**)	7/10(189**)	2.4**	2.8**
	100	5/5	1.1	0/10	6.4	0.7*	4/8(72442)	4/8(2441)	3.7	4.5
	50	5/5	0.2	6/10	7.0	1.0	9/10*(109**)	9/10*(1036)	1.0**	1.4**
Ribavirin[g]	350	5/5	0.9	10/10*	>21.0**	0.9	10/10**(48**)	10/10**(15**)	3.5	4.6
CMC	-	-	-	15/20	6.0	1.4	11/20(1151)	9/20(1472)	4.4	4.9
Normals	-	5/5	0.5	-	-	0.5	5/5(54)	5/5(9)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

[g] Ribavirin given 4 hr post-virus inoculation in H_2O.

Conclusions: AVS2778 (ABMP) was significantly effective vs PTV in vivo using single per os treatment. In this experiment, although an inadequate number of virus control mice died, liver scores, SGOT, SGPT, liver virus and serum virus were significantly reduced.

*P<0.05 **P<0.01

Table V-88. Expt. PtA333. Effect of Once Daily Per Os Treatment with AVS2778 on Punta Toro Virus Infections in Mice.

Animals: 11.0-12.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 3, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2778	400	5/5	-1.9	6/10**	7.0**	0.1**	10/10**(74**)	9/10**(24**)	1.8**	2.0**
	200	5/5	-0.6	3/10**	7.0**	0.2**	7/9**(322**)	7/9**(245**)	4.0**	4.6**
	100	5/5	0.0	0/10	5.5*	0.6**	0/7(4436*)	1/7(3504**)	5.7	6.3
	50	5/5	1.2	0/10	5.0	2.0	0/10(8410)	0/10(7616)	5.4	6.4
	25	5/5	1.4	1/10	4.9	1.2**	0/8(7006)	0/8(6243)	5.4	6.2
	12.5	5/5	1.1	0/10	5.0	1.2**	1/9(4039*)	1/9(3416**)	5.2	6.0
Ribavirin[g]	75	5/5	2.4	10/10**	>21.0**	0.8**	10/10**(67**)	10/10**(35**)	2.5**	4.3**
CMC	-	-	-	0/20	4.9	2.5	0/18(7327)	0/18(7225)	5.6	6.4
Normals	-	5/5	1.0	-	-	0.0	5/5(46)	5/5(10)	0.7	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Ribavirin administered qd x 5 beginning 24 hr pre-virus inoculation.

Conclusions: AVS2778 (ABMP) was significantly effective when used orally once daily for 3 days. This activity appears less pronounced than that exerted by AVS2776 (ABPP), however.

*P<0.05 **P<0.01

Table V-89. Expt. PtA500-502. Effect of Once Only i.p. Treatment With AVS2779 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.8-14.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2779	24 hr pre	100	5/5	0.2	9/10**	9.0
		50	5/5	0.3	10/10**	>21.0**
		25	5/5	0.6	10/10**	>21.0**
		12.5	5/5	0.6	6/10*	7.8*
		6.25	5/5	1.2	9/10**	8.0
	4 hr post	100	5/5	0.2	10/10**	>21.0**
		50	5/5	0.3	10/10**	>21.0**
		25	5/5	0.6	10/10**	>21.0**
		12.5	5/5	0.6	10/10**	>21.0**
		6.25	5/5	1.2	10/10**	>21.0**
	24 hr post	100	5/5	0.2	9/10**	5.0
		50	5/5	0.3	7/10**	5.3
		25	5/5	0.6	7/10**	5.0
		12.5	5/5	0.6	6/10*	5.3
		6.25	5/5	1.2	3/10	5.4
Ribavirin		350	5/5	0.1	10/10**	>21.0**
Saline		-	-	-	4/20	5.6
Normals		-	5/5	0.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS2779 (MVE-1), an apparent immunomodulator, was highly active vs PTV in this experiment.

Table V-90. Expt. PtA236. Effect of AVS2811 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 10.2-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2811	25	5/5	2.3	0/10	4.1
	12.5	5/5	2.8	3/10	7.1
	6.25	5/5	3.5	5/10	4.8
	3.13	5/5	2.5	5/10	7.4
Ribavirin	75	5/5	2.6	10/10*	>21.0**
Saline	-	-	-	14/20	5.0
Normals	-	5/5	3.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is 7-deoxynarciclasine, which *in vitro* had moderate activity vs PTV. The NCI reported it to be used in their testing at 12.5 mg/kg/day. No activity was seen in the present *in vivo* PTV experiment.

Table V-91. Expt. PtA369. Effect of Twice Daily i.p. Treatment With AVS2811 on Punta Toro Virus Infections in Mice.

Animals: 12.0-12.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

Compound	Dosage (mg/kg/day)	Tox. Control Surv/ Total	Infected, Treated Surv/ Total	Infected, Treated MST[a] (days)
AVS2811	8	5/5	2/10	5.0
	4	5/5	7/10**	7.7
	2	5/5	1/10	5.3
	1	5/5	2/10	5.3
Ribavirin[b]	75	5/5	10/10**	>21.0**
CMC	-	-	3/20	5.0
Normals	-	5/5	-	-

[a]Mean survival time of mice dying on or before day 21.

[b]Diluent is saline.

*P<0.05 **P<0.01

Conclusions: In this initial experiment, AVS2811, 7-deoxynarciclasine, was effective at a single dose against PTV in vivo.

Table V-92. Expt. PtA237. Effect of AVS2812 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.5-13.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2812	6	4/5	3.1	10/10*	>21.0**
	3	5/5	2.5	9/10	5.0
	1.5	5/5	2.1	7/10	5.3
	0.75	5/5	3.1	3/10	5.0
Ribavirin	75	5/5	2.6	10/10*	>21.0**
Saline	-	-	-	14/20	5.0
Normals	-	5/5	3.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is narciclasine, which *in vitro* had moderate activity vs PTV. The NCI reported an LD50 dose to be 5 mg/kg/day; we therefore selected the maximum dose to be 6 mg/kg/day, which was lethally toxic to 1 of 5 toxicity control mice, but the remaining mice surprisingly gained weight at a rate equivalent to normal mice. This dose was significantly effective in preventing death in the PTV-infected mice. The next dose prevented death in 90% of the mice. The data were somewhat compromised by the low death rate in the saline-treated virus controls. A second experiment is underway to confirm these data.

Table V-92B. Expt. PtA292. Effect of Once Daily Treatment with AVS2812 on Punta Toro Virus Infections in Mice (Confirming Experiment).

Animals: 13.5-14.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2812	12	5/5	3.3	6/10	8.3	0.0**	10/10**(28**)	10/10**(9**)	1.2**	2.9
	6	5/5	2.9	10/10	>21.0**	1.6	7/10(293)	2/10(251)	2.5	4.5
	3	5/5	3.4	10/10	>21.0**	0.7**	10/10**(39**)	10/10**(18**)	0.0**	1.8**
	1.5	5/5	1.7	10/10	>21.0**	0.7**	10/10**(74**)	9/10**(20**)	0.3**	1.4**
	0.75	5/5	3.4	10/10	>21.0**	1.0	5/10(620)	5/10(512)	4.1	5.1
Ribavirin	75	5/5	2.9	10/10	>21.0**	0.4**	10/10**(72**)	10/10**(18**)	0.3**	1.7
Saline	-	-	-	17/20	6.3	1.4	4/19(1161)	3/19(1197)	3.7	4.9
Normals	-	5/5	3.9	-	-	0.0	5/5(78)	5/5(25)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS2812 (narciclasine) was significantly effective in this experiment, confirming the activity seen initially in PtA 237 (Table V-92). The survival data were compromised, however, by the low numbers of saline-treated controls which died.

*P<0.05 **P<0.01

Table V-93. Expt. PtA206. Effect of Twice Daily i.p. Treatment with AVS2880 on Punta Toro Virus Infections in Mice.

Animals: 9.9-11.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 3, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2880	25	5/5	1.4	0/10	5.1
	12.5	5/5	1.5	0/10	5.7
	6.25	5/5	1.4	0/10	5.9*
	3.13	5/5	1.3	1/10	5.8*
	1.56	5/5	1.0	2/10*	5.9
	0.78	5/5	1.5	0/10	5.1
Ribavirin	75	5/5	2.8	9/10**	11.0
Saline	-	-	-	0/20	5.2
Normals	-	5/5	1.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is oxamisole, which has previously exhibited positive but erratic effects vs PTV *in vivo*. This study was run to further elucidate the best treatment regimen for this immune modulating substance. Positive but again erratic activity was seen, with doses of 6.25, 3.13 and 1.56 mg/kg/day exerting those positive effects. The 1.56 mg/kg/day dosage was shown earlier to also be effective when given once daily for 3 days (PtA 82).

Table V-94. Expt. PtA258. Effect of Two Daily Treatments with AVS2880 on Punta Toro Virus Infections in Mice [Treatment Begun 24 hr Pre-virus Inoculation].

Animals: 10.6-12.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 2, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2880	50	4/5	1.6	4/10	5.8
	25	5/5	0.3	4/10	6.0
	12.5	5/5	0.9	1/10	5.4
	6.25	5/5	0.4	3/10	6.0
	3.13	5/5	1.0	2/10	5.6
	1.56	5/5	0.2	0/10	6.3
	0.78	5/5	0.4	8/10**	5.5
Ribavirin	38	5/5	2.1[c]	10/10**	>21.0**
Saline	-	-	-	6/20	5.9
Normals	-	5/5	0.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Weight 3 days later.

*P<0.05 **P<0.01

Conclusions: This experiment is another in a series run to determine the most appropriate treatment regimen for AVS2880 (oxamisole). Significant activity was seen only at the lowest dosage used, a result not too surprising in light of the previously observed erratic activity of this immunemodulator.

Table V-95. Expt. PtA268. Effect of Two Daily Treatment with AVS2880 on Punta Toro Virus Infections in Mice [Treatment Begun 4 hr Pre-virus Inoculation].

Animals: 10.6-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 2, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2880	50	5/5	0.1	5/10	4.4
	25	5/5	0.4	6/10	5.8
	12.5	5/5	0.7	4/10	4.8
	6.25	5/5	0.2	2/10	4.4
	3.13	5/5	0.7	6/10	5.8
	1.56	5/5	0.5	4/10	4.7
	0.78	5/5	0.6	3/10	5.9
Ribavirin[c]	350	5/5	0.6	9/10**	9.0
Saline	-	-	-	7/20	5.2
Normals	-	5/5	1.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered once only 4 hr post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: Two i.p. treatments with AVS2880 (oxamisole) beginning 4 hr pre-virus inoculation were less effective than those beginning 24 hr pre-virus inoculation (see PtA 258).

Table V-96. Expt. PtA269. Effect of Two Daily Treatments with AVS2880 on Punta Toro Virus Infections in Mice [Treatment Begun 4 hr Post-virus Inoculation].

Animals: 10.6-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 2, 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2880	50	5/5	0.1	8/10*	4.0
	25	5/5	0.4	9/10**	5.0
	12.5	5/5	0.7	6/10	4.8
	6.25	5/5	0.2	5/10	4.8
	3.13	5/5	0.7	4/10	4.3
	1.56	5/5	0.5	3/10	4.9
	0.78	5/5	0.6	1/10	4.6
Ribavirin[c]	350	5/5	0.6	9/10**	9.0
Saline	-	-	-	7/20	5.2
Normals	-	5/5	1.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered once only 4 hr post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: Two i.p. treatments with AVS2880 (oxamisole) beginning 4 hr post-virus inoculation were more effective than those beginning 24 hr pre, 4 hr pre, 24 hr post or 48 hr post-virus inoculation (compare with PtA 258, 268, 270, 271).

Table V-97. Expt. PtA270. Effect of Two Daily Treatments with AVS2880 on Punta Toro Virus Infections in Mice [Treatment Begun 24 hr Post-virus Inoculation].

Animals: 10.6-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 2, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2880	50	5/5	0.1	1/10	5.1
	25	5/5	0.4	5/10	5.8
	12.5	5/5	0.7	5/10	5.6
	6.25	5/5	0.2	5/10	5.6
	3.13	5/5	0.7	5/10	5.0
	1.56	5/5	0.5	5/10	6.0
	0.78	5/5	0.6	3/10	4.4
Ribavirin[c]	350	5/5	0.6	9/10**	9.0
Saline	-	-	-	7/20	5.2
Normals	-	5/5	1.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered once only 4 hr post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: Two i.p. treatments with AVS2880 (oxamisole) beginning 24 hr post-virus inoculation were less effective than those beginning 24 hr pre-virus inoculation (see PtA 258).

Table V-98. Expt. PtA271. Effect of Two Daily Treatments with AVS2880 on Punta Toro Virus Infections in Mice [Treatment Begun 48 hr Post-virus Inoculation].

Animals: 10.6-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.

Treatment Schedule: Once daily x 2, 48 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2880	50	5/5	0.1	6/10	4.5
	25	5/5	0.4	1/10	4.4
	12.5	5/5	0.7	4/10	5.7
	6.25	5/5	0.2	5/10	4.8
	3.13	5/5	0.7	5/10	4.6
	1.56	5/5	0.5	8/10*	5.0
	0.78	5/5	0.6	3/10	5.3
Ribavirin[c]	350	5/5	0.6	9/10**	9.0
Saline	-	-	-	7/20	5.2
Normals	-	5/5	1.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered once only 4 hr post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: Two i.p. treatments with AVS2880 (oxamisole) beg 48 hr post-virus inoculation were essentially as effective as those beginning 24 hr pre-virus inoculation (compare PtA 258, 268, 269, 270).

Table V-99. Expt. PtA272. Effect of Every Three Day Treatment with AVS2880 on Punta Toro Virus Infections in Mice.

Animals: 10.6-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Every 3 days x 3, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2880	50	5/5	4.0	3/10	4.3
	25	5/5	4.0	3/10	5.4
	12.5	5/5	3.7	5/10	6.6
	6.25	5/5	4.1	3/10	6.9
	3.13	5/5	3.8	2/10	5.8
	1.56	5/5	5.0	6/10	5.3
	0.78	5/5	3.6	0/10	5.4
Ribavirin[c]	350	5/5	0.6	9/10**	9.0
Saline	-	-	-	7/20	5.2
Normals	-	5/5	1.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered once only 4 hr post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: Treatment i.p. every 3 days with AVS2880 (oxamisole) were ineffective vs PTV *in vivo*.

Table V-100. Expt. PtA334. Effect of Once Daily Per Os Treatment with AVS2880 on Punta Toro Virus Infections in Mice.

Animals: 11.0-12.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once daily x 2, beginning 4 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2880	50	5/5	1.1	0/10	4.8**	1.5	1/9(6800)	1/9(6586)	5.8	6.5
	25	5/5	1.2	0/10	4.2*	1.1	0/8(4432)	0/8(4330)	5.5	6.0
	12.5	5/5	1.2	0/10	4.8**	0.9	1/6(5117)	1/6(4365)	5.7	6.4
	6.25	5/5	1.1	0/10	4.2*	1.1	2/10(3736)	0/10(4706)	5.2	5.7
	3.13	5/5	1.4	0/10	3.9	1.0	3/10(4259)	2/10(4946)	5.3	5.8
	1.56	5/5	0.7	0/10	4.1	0.9	0/8(4435)	0/9(5084)	6.1	6.4
	0.76	5/5	0.7	2/10	4.8*	0.8	0/10(4946)	0/10(5022)	5.6	6.3
Ribavirin[g]	75	5/5	2.4	10/10**	>21.0**	0.8	10/10**(67**)	10/10**(35**)	2.5**	4.3**
H_2O	-	-	-	1/20	3.8	0.8	4/19(2817)	3/19(3426)	5.9	5.5
Normals	-	5/5	0.7	-	-	0.0	5/5(68)	5/5(40)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Ribavirin administered qd x 5 beginning 24 hr pre-virus inoculation. Also weighed one day later for final weight.

Conclusions: Oxamisole has continued to show erratic activity vs PTV in vivo. In this study, two p.o. treatments were only moderately effective as seen by increased mean survival times.

*P<0.05 **P<0.01

Table V-101. Expts. PtA350-355. Effect of Single i.p. Treatments with AVS2933 at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.4-13.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Ca^{++}, Mg^{++} free Saline.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control			Infected, Treated	
Compound	Time of Treatment	Dosage (ug/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2933	48 hr pre	1000	5/5	-0.2	2/10*	6.6
		100	5/5	-0.2	1/10	6.1
		10	5/5	-0.2	0/10	6.6
	24 hr pre	1000	5/5	-0.2	0/10	5.6
		100	5/5	-0.2	2/10*	6.6
		10	5/5	-0.2	2/10*	5.4
	4 hr pre	1000	5/5	-0.2	0/10	4.8
		100	5/5	-0.2	1/10	5.3
		10	5/5	-0.2	0/10	4.5
	24 hr post	1000	5/5	-0.2	5/10**	5.4
		100	5/5	-0.2	2/10*	6.8
		10	5/5	-0.2	1/10	4.3
	48 hr post	1000	5/5	-0.2	0/10	5.3
		100	5/5	-0.2	1/10	4.4
		10	5/5	-0.2	0/10	4.6
	72 hr post	1000	5/5	-0.2	3/10*	5.0
		100	5/5	-0.2	0/10	4.5
		10	5/5	-0.2	0/10	4.4
Ribavirin	24 hr post	350[c]	5/5	-0.3	10/10**	>21.0**
Saline	24 hr post	-	-	-	0/20	7.1
Normals		-	5/5	0.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]mg/kg/day.

*P<0.05 **P<0.01

Conclusions: CGP 19835 (liposome monomer tripeptide) was considered moderately effective vs PTV in this series of experiments. Best activity appeared to occur when the compound was given 24 hr after virus exposure. Some erraticism was seen in these experiments with this immune modulating substance, a not unusual observation with such materials.

Table V-102. Expt. PtA402. Effect of Every Other Day i.p. Treatment With AVS2933 on Punta Toro Virus Infections in Mice.

Animals: 10.7-13.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Ca^H, Mg^H free saline.
Treatment Schedule: Every other day x 3, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (µg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2933	1000	5/5	2.5	1/10	6.1
	100	5/5	2.0	0/10	6.2
	10	5/5	2.1	0/10	5.5
	1	5/5	2.3	0/10	5.2
Saline	-	-	-	1/20	5.7
Normals	-	5/5	2.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: CGP 19835A (liposome monomer tripeptide) was ineffective vs PTV when administered i.p. every other day in this study.

Table V-103. Expt. PtA410. Effect of Once Only i.p. Treatment with AVS2933 on Punta Toro Virus Infections in Mice.

Animals: 12.3-13.6g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Ca^{++}, Mg^{++} free saline.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (ug/kg)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2933	10,000	5/5	0.6	7/10**	8.3**	1.8**	1/9(1711**)	0/9(1775**)	2.3**	4.5**
	5,000	5/5	1.1	10/10**	>21.0**	2.2**	0/10(1300**)	0/10(1176**)	3.3**	4.3**
	2,500	5/5	1.0	4/10*	6.0	2.5	0/10(3609**)	0/10(2478**)	3.9	4.2**
	1,250	5/5	1.8	9/10**	4.0	2.7	0/10(6985**)	0/10(5115**)	4.5	4.6**
	625	5/5	1.2	0/10	5.1	2.4*	0/9(6917**)	0/9(4783**)	3.5	4.3**
	313	5/5	1.3	3/10	4.6	2.0**	0/10(8845)	0/10(6170**)	3.9	4.7**
Ribavirin	350[g]	5/5	0.5	nr[h]	nr[h]	0.7**	3/10*(277**)	10/10**(55**)	1.6**	0.4**
Saline	-	-	-	1/20	4.7	2.7	0/20(12735)	0/20(9698)	4.7	5.8
Normals	-	5/5	2.3	-	-	0.2	5/5(121)	5/5(56)	0.0	0.0
AVS4726[i]	undilute	5/5	0.7	0/10	4.8	3.0	0/8(10,525)	0/8(10,050)	5.7	6.3
Ribavirin[i]	350	5/5	0.2	9/10**	10.0	1.2**	1/9(2915**)	1/9(3267**)	4.3**	4.6**
Saline[i]	-	-	-	0/20	4.5	3.1	0/18(11,057)	0/18(11,044)	5.5	6.2
Normals[i]	-	5/5	0.7	-	-	0.0	4/5(181)	5/5(54)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Ribavirin dosage is mg/kg.

[h]Not run by mistake.

[i]These were run at a later date as experiment PtA462. AVS4726 is the placebo for AVS2933.

Conclusions: CGP19835A (liposome monomer tripeptide) was highly active vs PTV in this study, repeating and expanding the results seen previously (PtA 353). In a separately run experiment, the placebo for this immunomodulator was, predictably, not effective.

*P<0.05 **P<0.01

Table V-104. Expt. PtA298. Effect of AVS2978 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 13.9-14.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2978	200	5/5	3.2	3/10	6.0
	100	5/5	3.8	0/10	5.4
	50	5/5	4.1	7/10*	5.3
	25	5/5	3.7	3/10	5.0
Ribavirin	75	5/5	2.9	10/10**	>21.0**
CMC	-	-	-	6/20	6.0
Normals	-	5/5	3.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this experiment, AVS2978 (the tetracetate ester of 2980; structurally related to 7-deoxynarciclasine [AVS2811]) was effective vs PTV at the 50 mg/kg/day dosage level only; this effect is unusual, since that dose is not approaching the maximum tolerated, unless the compound is exhibiting an immunomodulating effect. It should be pointed out that AVS2978 is highly insoluble, so there may have been problems with its absorption. This experiment should be repeated to verify these data.

Table V-105. Expt. PtA332. Effect of Once Daily i.p. Treatment With AVS2978 on Punta Toro Virus Infections in Mice.

Animals: 11.0-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, 36 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2978	400	5/5	1.7	0/10	4.5
	200	5/5	3.4	0/10	3.9
	100	5/5	2.1	0/10	3.8
	50	5/5	2.6	0/10	4.5
	25	5/5	2.1	0/10	4.8
Ribavirin	75	5/5	2.8	7/10**	9.3**
CMC	-	-	-	0/20	4.0
Normals	-	5/5	4.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound is the tetraacetate ester of AVS2980, structurally related to 7-deoxynarciclasine (AVS2811). We previously found it to be active vs PTV at 50 mg/kg/day only (PTA 298). Higher doses were ineffective. In this present study, the experiment was repeated starting with a higher dosage level. This dosage (400 mg/kg/day), while well tolerated, caused a lesser weight gain in toxicity controls than the lower dosages, suggesting it was approaching the MTD. No activity was seen at any dose.

Table V-106. Expt. PtA266. Effect of Twice Daily i.p. Treatment with AVS2980 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 10.1-12.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2980	500	0/5	-	0/10	1.0
	250	0/5	-	0/10	1.2
	125	0/5	-	0/9	3.4
	62.5	1/5	0.4	0/9	5.6
	31.3	4/5	0.5	1/9	4.5
Ribavirin	75	5/5	1.3	10/10**	>21.0**
CMC	-	-	-	12/20	5.6
Normals	-	5/5	3.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is the tetrahydroxy analogue of pancratistatin. It had moderate activity vs PTV *in vitro*. Preliminary USAMRIID data indicated this compound was not toxic up to 124 mg/kg/day. In the present study, however, toxicity was seen at all doses, including 31.3 mg/kg/day. We predict the maximum tolerated dose to be approximately 15 mg/kg/day. No activity vs PTV was seen in this study, but this would be expected in light of the toxicity manifested. The experiment is being repeated at lower dosages.

Table V-107. Expt. PtA299. Effect of Twice Daily i.p. Treatment with AVS2980 on Punta Toro Virus Infections in Mice (Repeat of Initial Test).

Animals: 13.9-15.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2980	15	5/5	2.8	6/10	5.0
	7.5	5/5	2.3	6/10	5.8
	3.75	5/5	3.3	6/10	5.3
	1.875	5/5	3.6	6/10	6.0
Ribavirin	75	5/5	2.9	10/10**	>21.0**
CMC	-	-	-	6/20	6.0
Normals	-	5/5	3.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound is a tetrahydroxy analog related structurally to 7-deoxynarciclasine (AVS360). No activity was seen in this experiment.

Table V-108. Expt. PtA396-398. Effect of Once Only i.p. Treatment With AVS2980 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.5-13.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS2980	4 hr pre	50	5/5	-0.5	3/10	5.0
		25	5/5	-1.9	0/10	5.3
		12.5	5/5	0.1	0/10	4.6
		6.25	5/5	0.3	1/10	4.8
	4 hr post	50	5/5	-0.5	5/10	4.6
		25	5/5	-1.9	6/10	7.5
		12.5	5/5	0.1	1/10	5.3
		6.25	5/5	0.3	2/10	4.9
	24 hr post	50	5/5	-0.5	3/10	6.4
		25	5/5	-1.9	0/10	4.7
		12.5	5/5	0.1	0/10	4.8
		6.25	5/5	0.3	1/10	6.0
Ribavirin		350	5/5	-0.2	10/10**	>21.0**
CMC		-	-	-	8/20	5.3
Normals		-	5/5	0.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is the tetrahydroxy analog of pancratistatin. Single i.p. treatment was considered not effective in this experiment at any treatment period used.

Table V-109. Expt. PtA451. Effect of Twice Daily i.p. Treatment With AVS3425 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.4-12.4 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3425	500	3/5	-1.9	0/10	3.9
	250	5/5	-1.0	0/10	4.4
	125	4/5	0.5	0/10	4.1
	62.5	5/5	0.9	0/10	4.0
	31.3	5/5	2.0	3/10	4.4
	15.6	5/5	3.4	3/10	4.3
Ribavirin	75	5/5	2.3	10/10**	>21.0**
CMC	-	-	-	1/20	4.5
Normals	-	5/5	3.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3425 (8-bromoguanosine) was inactive vs PTV in this experiment.

Table V-110. Expt. PtA491-493. Effect of Once Only s.c. Treatment With AVS3425 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.8-15.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3425	24 hr pre	250	5/5	-1.8	0/10	6.3
		125	5/5	-1.7	2/10	6.1
		62.5	5/5	0.2	1/10	6.4
		31.3	5/5	0.2	5/10	5.8
		15.6	5/5	-0.2	3/10	6.1
	4 hr post	250	5/5	-1.8	0/10	4.8
		125	5/5	-1.7	0/10	6.1
		62.5	5/5	0.2	0/10	5.2
		31.3	5/5	0.2	3/10	5.7
		15.6	5/5	-0.2	6/10	5.8
	24 hr post	250	5/5	-1.8	0/10	4.2
		125	5/5	-1.7	0/10	4.3
		62.5	5/5	0.2	0/10	4.2
		31.3	5/5	0.2	2/10	4.8
		15.6	5/5	-0.2	2/10	4.9
Ribavirin		350	5/5	-0.9	10/10*	>21.0**
CMC		-	-	-	11/20	6.1
Normals		-	5/5	-0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3425 (8-bromoguanosine) was ineffective in this experiment.

Table V-111. Expt. PtA505. Effect of Once Daily s.c. Treatment With AVS3425 on Punta Toro Virus Infections in Mice.

Animals: 13.2-14.0 g (4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3425	200	5/5	-1.5	1/10	4.4
	100	5/5	-0.1	1/10	5.2
	50	5/5	2.0	3/10	6.1**
	25	5/5	2.7	2/10	5.3
Ribavirin	75	5/5	1.1	10/10**	>21.0**
CMC	-	-	-	2/20	4.8
Normals	-	5/5	2.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3425 (8-bromoguanosine) was moderately effective vs PTV using this treatment regimen.

Table V-112. Expt. PtA506-508. Effect of Once Only s.c. Treatment With AVS3425 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 13.0-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3425	24 hr pre	400	4/5	-1.7	1/10	5.2
		200	5/5	-1.5	0/10	5.3
		100	5/5	-0.5	2/10	6.9
		50	5/5	-0.2	0/10	5.5
	4 hr post	400	4/5	-1.7	0/10	5.9
		200	5/5	-1.5	5/10*	6.0
		100	5/5	-0.5	1/10	6.0
		50	5/5	-0.2	1/10	5.9
	24 hr post	400	4/5	-1.7	0/10	4.2
		200	5/5	-1.5	1/10	4.3
		100	5/5	-0.5	0/10	4.4
		50	5/5	-0.2	0/10	4.8
Ribavirin		350	5/5	0.0	10/10**	>21.0**
CMC		-	-	-	3/20	6.6
Normals		-	5/5	0.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3425 (8-bromoguanosine) was active vs PTV only at a single dosage given 4 hr post-virus inoculation.

Table V-113. Expt. PtA525. Effect of Twice Daily p.o. Treatment With AVS3425 on Punta Toro Virus Infections in Mice.

Animals: 9.3-12.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3425	250	5/5	3.5	0/10	5.2
	125	5/5	4.0	0/10	5.1
	62.5	5/5	4.0	0/10	5.8
	31.3	5/5	4.7	0/10	5.5
	15.6	5/5	2.1	0/10	5.8
Ribavirin	75	5/5	2.8	10/10**	>21.0**
CMC	-	-	-	1/20	6.0
Normals	-	5/5	5.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3425 (8-bromoguanosine) was ineffective vs PTV using this oral treatment regimen.

Tale V-114. Expt. PtA526-528. Effect of Once Only s.c. Treatment With AVS3425 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.6-14.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3425	24 hr pre	800	4/5	-1.6	0/10	5.8**
		400	5/5	-1.4	0/10	5.6**
		200	5/5	-0.1	2/10*	5.9**
		100	5/5	0.4	1/10	6.3**
	4 hr post	800	4/5	-1.6	7/10**	5.3
		400	5/5	-1.4	0/10	5.4*
		200	5/5	-0.1	5/10**	5.8*
		100	5/5	0.4	0/10	5.8*
	24 hr post	800	4/5	-1.6	2/10*	5.1
		400	5/5	-1.4	0/10	5.1
		200	5/5	-0.1	0/10	4.9
		100	5/5	0.4	1/10	4.9
Ribavirin		350	5/5	0.1	10/10**	>21.0**
CMC		-	-	-	0/20	4.5
Normals		-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3425 (8-bromoguanosine) was relatively active vs PTV in this study. These data confirm the activity seen in PtA 507.

Table V-115. Expt. PtA404. Effect of Twice Daily i.p. Treatment With AVS3580 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.8-13.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3580	100	5/5	3.1	0/10	4.1
	50	5/5	3.6	1/10	4.3
	25	5/5	3.3	2/10*	4.5
	12.5	5/5	3.1	0/10	4.6
	6.25	5/5	3.3	0/10	4.1
Ribavirin	75	5/5	3.3	10/10**	>21.0**
Saline	-	-	-	0/20	4.4
Normals	-	5/5	4.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This unidentified compound, defined only as a non-immunomodulator, was not effective vs PTV in this initial study. The material was well tolerated at all doses used, however, suggesting a repeated experiment with higher dose levels.

Table V-116. Expt. PtA532-533. Effect of Once Only s.c. Treatment With AVS3580 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.2-13.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (µg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3580	24 hr pre	300	5/5	0.1	1/10	6.6**
		150	5/5	0.6	0/10	6.9**
		75	5/5	0.5	3/10	5.7
		37.5	5/5	0.3	4/10	6.7*
	4 hr post	300	5/5	0.1	0/10	5.0
		150	5/5	0.6	2/10	5.6
		75	5/5	0.5	0/10	4.4
		37.5	5/5	0.3	0/10	5.2
Ribavirin		350	5/5	0.1	7/10**	6.7
Saline		-	-	-	1/20	5.0
Normals		-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This unidentified compound was moderately active vs PTV in this experiment where mice were pretreated with a single dosage of the compound. A toxic dose was not yet achieved with the material.

Table V-117. Expt. PtA316. Effect of Once Only Oral Therapy With AVS3585 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3585	24	5/5	-0.2	2/10	5.6
	12	5/5	0.6	0/10	6.1
	6	5/5	0.6	0/10	5.5
	3	5/5	-0.2	2/10	5.9
Ribavirin	350	5/5	-0.3	2/10	6.9*
H_2O	-	-	-	1/20	5.4
Normals	-	5/5	0.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Neurotropin, used orally in this study, was inactive vs PTV.

Table V-118. Expt. PtA399-401. Effect of Once Only i.p. Treatment With AVS3587 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.1-13.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3587	4 hr pre	400	5/5	0.1	0/10	5.0
		200	5/5	0.4	2/10	6.4
		100	5/5	0.2	0/10	5.5
		50	5/5	0.4	1/10	5.0
	4 hr post	400	5/5	0.1	10/10**	>21.0**
		200	5/5	0.4	9/10**	5.0
		100	5/5	0.2	8/10*	6.5
		50	5/5	0.4	5/10	6.6
	24 hr post	400	5/5	0.1	6/10	5.5
		200	5/5	0.4	2/10	6.3
		100	5/5	0.2	9/10**	8.0
		50	5/5	0.4	8/10*	6.0
Ribavirin		350	5/5	-0.2	10/10**	>21.0**
CMC		-	-	-	8/20	5.3
Normals		-	5/5	0.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is the Upjohn immunomodulator 2-amino-5-chloro-6-phenyl-4(3H)-pyrimidinone (ACPP). Single i.p. treatment was highly effective when administered post-virus inoculation.

Table V-119. Expt. PtA435. Effect of Single i.p. Treatment with AVS3587 on Punta Toro Virus Infections in Mice.

Animals: 12.0-13.3 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3587	500	5/5	0.4	4/10	7.0	2.0	2/10*(3335)	3/10*(2825)	3.0	3.5
	250	5/5	0.1	3/10	6.4	2.8	1/10(3642)	0/10(3335)	2.8	4.1
	125	5/5	0.6	6/10	9.0	2.2	1/10(1230*)	1/10(1643)	4.2	4.6
	62.5	4/4	0.8	1/10	6.1	2.8	2/9*(2367)	0/9(1951)	1.8	2.9
	31.3	5/5	0.2	1/10	6.3	2.9	0/10(1081**)	0/10(1280**)	3.1	4.5
Ribavirin	350	5/5	-0.5	8/10	6.5	0.4**	6/10**(232**)	9/10**(110**)	2.3	3.3
CMC	-	-	-	12/20	5.5	2.1	0/19(4259)	0/19(3609)	2.7	3.5
Normals	-	5/5	1.0	-	-	0.0	1/5(344)	5/5(74)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This is the Upjohn immunomodulator 2-amino-5-chloro-6-phenyl-4(3H)-pyrimidinone (ACPP), which in a previous study was highly active vs PTV using this treatment schedule. In the present, confirming, experiment, however, essentially no activity was seen. The study will be repeated.

*P<0.05 **P<0.01

Table V-120. Expt. PtA457. Effect of Single p.o. Treatment with AVS3587 on Punta Toro Virus Infections in Mice.

Animals: 11.8-13.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3587	500	5/5	-0.4	2/10*	4.8	2.2	0/9(9404**)	0/9(8396*)	5.3	6.1
	250	5/5	0.2	1/10	5.3	3.2	0/10(15,025)	0/10(10,495)	5.8	6.3
	125	5/5	0.4	0/10	5.0	1.7**	0/8(8494**)	0/8(7788**)	5.6	6.1
	62.5	5/5	0.4	0/10	4.7	2.8	0/9(6388**)	0/9(4849**)	4.8	5.1
	31.3	5/5	0.1	0/10	5.1	2.9	0/8(11,081)	0/8(10,000)	5.6	6.2
Ribavirin	350	5/5	0.2	9/10**	10.0	1.2**	1/9(2915**)	1/9(3267**)	4.3**	4.6**
CMC	-	-	-	0/20	4.7	3.0	0/17(17,333)	0/17(11,903)	5.7	6.1
Normals	-	5/5	0.7	-	-	0.0	4/5(181)	5/5(54)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Previous studies with AVS3587 (2-amino-5-chloro-6-phenyl-4(3H)-pyrimidinone) indicated it was highly active vs PTV in vivo when administered i.p. in single injections. Oral gavage therapy was only moderately effective in this experiment.

*P<0.05 **P<0.01

Table V-121. Expt. PtA318-323. Effect of Varying Times of Single i.p. Treatment with AVS3563 on Punta Toro Virus Infections in Mice.

Animals: 11.6-12.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: Once only, varying times relative to virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3588	4 hr pre	400	5/5	-0.2	9/10**	6.0
		200	5/5	0.6	7/10**	4.3
		100	5/5	0.6	7/10**	4.7
		50	5/5	0.4	5/10**	6.6
	4 hr post	400	5/5	-0.2	10/10**	>21.0**
		200	5/5	0.6	9/10**	4.0
		100	5/5	0.6	10/10**	>21.0**
		50	5/5	0.4	8/10**	5.0
	24 hr post	400	5/5	-0.2	10/10**	>21.0**
		200	5/5	0.6	10/10**	>21.0**
		100	5/5	0.6	10/10**	>21.0**
		50	5/5	0.4	10/10**	>21.0**
	48 hr post	400	5/5	-0.2	0/10	4.6
		200	5/5	0.6	0/10	4.7
		100	5/5	0.6	1/10	5.7
		50	5/5	0.4	0/10	5.7
	72 hr post	400	5/5	-0.2	0/10	4.1
		200	5/5	0.6	4/10*	5.2
		100	5/5	0.6	1/10	4.8
		50	5/5	0.4	2/10	4.1
	96 hr post	400	5/5	-0.2	0/10	4.3
		200	5/5	0.6	0/10	4.3
		100	5/5	0.6	1/10	4.6
		50	5/5	0.4	0/10	4.5
Ribavirin	24 hr post	350	5/5	-0.3	2/10	6.9*
CMC	24 hr post	-	-	-	1/20	5.4
Normals		-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3588 is the metafluoro derivative of ABPP (AVS2776). In this experiment, varying times of single i.p. treatment were studied, with efficacy seen when given as late as 24 hr post-virus inoculation.

Table V-122. Expts. PtA344-348. Effect of Single i.p. Treatment with AVS3588 at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 9.3-10.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3588	4 hr pre	300	5/5	-0.4	5/10*	5.4
		150	5/5	0.1	4/10	4.7
		75	5/5	0.5	6/10**	5.8
		37.5	5/5	1.2	0/10	5.2
	4 hr post	300	5/5	-0.4	6/10**	5.8
		150	5/5	0.1	1/10	4.9
		75	5/5	0.5	3/10	6.3**
		37.5	5/5	1.2	2/10	4.6
	24 hr post	300	5/5	-0.4	10/10**	>21.0**
		150	5/5	0.1	8/10**	4.5
		75	5/5	0.5	0/10	4.7
		37.5	5/5	1.2	2/10	6.9**
	48 hr post	300	5/5	-0.4	0/10	4.4
		150	5/5	0.1	0/10	4.0
		75	5/5	0.5	0/10	4.0
		37.5	5/5	1.2	1/10	4.4
	72 hr post	300	5/5	-0.4	0/10	4.0
		150	5/5	0.1	1/10	4.3
		75	5/5	0.5	0/10	4.3
		37.5	5/5	1.2	0/10	4.0
Ribavirin	24 hr post	350	5/5	0.0	8/10**	5.5
CMC	24 hr post	-	-	-	2/20	4.6
Normals		-	5/5	0.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Metafluoro ABPP was moderately active vs PTV when administered as late as 24 hr post-virus exposure.

Table V-123. Expt. PtA458. Effect of Once Daily p.o. Treatment with AVS3589 on Punta Toro Virus Infections in Mice.

Animals: 11.5-12.4 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily, 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3589	500	5/5	0.4	2/10	6.6*	4.0	0/10(5475**)	0/10(4967**)	4.4**	4.3**
	250	5/5	0.0	0/10	6.6	2.3**	1/9(5996**)	0/9(5597**)	4.7*	5.3
	125	5/5	1.2	0/9	5.7	3.2	0/7(15,843)	0/7(12,500)	5.9	6.4
	62.5	5/5	0.9	1/10	6.1	3.4	0/9(9109)	0/9(6978*)	5.6	5.5
	31.3	5/5	0.8	0/10	5.8	3.2	0/7(9814)	0/7(9136)	5.6	6.2
Ribavirin	350	5/5	0.2	9/10**	10.0	1.2**	1/9(2915**)	1/9(3267**)	4.3**	4.6**
CMC	-	-	-	2/20	5.7	3.5	0/16(12,102)	0/16(11,103)	5.6	6.0
Normals	-	5/5	1.0	-	-	0.2	5/5(73)	5/5(34)	0.1	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Previous studies with AVS3589 (2-amino-5-chloro-2,3-difluorophenyl-4(3H)-pyrimidinone) indicated it had a moderate anti-PTV activity when used i.p. by this treatment schedule. Oral gavage therapy was considered less effective than the previous i.p. therapy in this experiment.

*P<0.05 **P<0.01

Table V-124. Expt. PtA389. Effect of Thrice Daily i.p. Treatment With AVS3593 on Punta Toro Virus Infections in Mice.

Animals: 11.6-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Thrice daily x 6, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3593	150	5/5	1.0	3/10	7.6
	75	5/5	1.6	0/10	6.2
	37.5	4/5	2.1	3/10	7.0
	18.8	5/5	2.8	1/10	5.4
	9.4	5/5	2.3	0/10	6.1
	4.7	5/5	2.0	5/10	6.2
	2.3	5/5	1.4	1/10	6.4
	1.2	5/5	2.2	5/10	7.4
Ribavirin	75	5/5	1.7	10/10**	>21.0**
Saline	-	-	-	4/20	6.1
Normals	-	5/5	2.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is the Lilly immune modulator Ly 253,963. In this experiment, using a thrice daily treatment schedule as recommended to us, no antiviral activity was seen. The material was well tolerated at all dosage levels, but the animals were gaining less weight at the highest dose, indicating the MTD was being approached.

Table V-125. Expt. PtA390. Effect of Twice Daily i.p. Treatment With AVS3593 on Punta Toro Virus Infections in Mice.

Animals: 10.8-13.4 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 6, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3593	150	5/5	1.4	1/10	5.6
	75	5/5	3.1	0/10	6.1
	37.5	4/5	2.4	1/10	6.2
	18.8	5/5	3.2	1/10	5.6
	9.4	5/5	1.9	0/10	5.8
	4.7	5/5	3.0	5/10	7.4
	2.3	5/5	1.9	4/10	6.0
	1.2	5/5	2.3	2/10	7.0
Ribavirin	75	5/5	1.7	10/10**	>21.0**
Saline	-	-	-	5/20	6.2
Normals	-	5/5	2.9	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is the Lilly immune modulator Ly 253,963. In this experiment, using a twice daily treatment schedule as recommended to us, no antiviral activity was seen. The material was well tolerated at all dosage levels, but the animals were gaining less weight at the highest dose, indicating the MTD was being approached.

Table V-126. Expt. PtA459-461. Effect of Once Only i.p. Treatment With AVS3593 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.7-13.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3593	24 hr pre	500	5/5	0.1	0/10	5.7**
		250	5/5	-0.4	0/10	5.5**
		125	5/5	-0.1	0/10	5.4**
		62.5	5/5	-0.8	0/10	6.1**
		31.3	5/5	-0.6	0/10	5.4**
	4 hr post	500	5/5	0.1	0/10	5.8*
		250	5/5	-0.4	0/9	4.2
		125	5/5	-0.1	0/10	4.4
		62.5	5/5	-0.8	0/10	4.7
		31.3	5/5	-0.6	1/10	5.7*
	24 hr post	500	5/5	0.1	0/10	4.6
		250	5/5	-0.4	0/10	5.5
		125	5/5	-0.1	0/10	4.4
		62.5	5/5	-0.8	1/10	5.6**
		31.3	5/5	-0.6	3/10*	5.7*
Ribavirin	24 hr post	350	5/5	-0.1	1/10	8.4**
Saline		-	-	-	0/20	4.7
Normals		-	5/5	-0.3	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3593 (Ly 253,963) was not effective previously when given i.p. three times daily or twice daily for 6 days (PtA 389, 390). Single i.p. treatments were of moderate efficacy vs PTV in this study, particularly if give 24 hr prior to virus inoculation. Ribavirin was surprisingly low in activity also, however, suggesting a need to repeat the study.

Table V-127. Expt. PtA499. Effect of *Ad Libitum* p.o. Treatment with AVS3593 on Punta Toro Virus Infections in Mice.

Animals: 10.9-13.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Drinking water.
Treatment Schedule: *Ad lib* in drinking water x 7, beg. 4 hr pre-virus inocul.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls			Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Mean H_2O Consum.[g]	Surv/ Total	MST[b] (days)	SGOT Mean Liver Score[c]	SGPT Neg/Total[d] (Mean)	Mean Liver Neg/Total[e] (Mean)	Mean Serum Virus Titer[f] (log_{10})	Virus Titer[f] (log_{10})
AVS3593	93	3/3	1.6	3.1	0/10	5.5	3.9	0/10(14,195)	0/10(12,310)	6.1	6.5
	45	3/3	3.8	4.1	0/10	6.1*	3.8	0/9(13,526)	0/9(11,350)	5.8	6.3
	25.5	3/3	3.1	3.9	1/10	5.7	3.4	0/8(12,216)	0/8(10,500)	5.8	6.0
	13.2	3/3	3.4	4.3	0/10	6.0**	3.1	0/10(9972)	0/10(8724)	5.5	6.2
	8.3	3/3	4.5	4.9	2/10*	5.9	2.8	1/10(5677*)	1/10(4881*)	3.6	3.8**
	3.6	3/3	4.3	4.6	0/10	5.5	3.9	0/10(8748)	0/10(7260)	5.1	
	1.8	3/3	4.5	5.1	2/10*	7.1**	3.7	0/10(7107)	0/10(6138)	4.6	5.4
	0.96	3/3	5.0	4.6	0/10	5.4	3.2	0/7(3336**)	0/7(3694**)	1.4**	4.3**
Ribavirin[h]	75	5/5	2.5	4.0	10/10**	>21.0**	0.1**	6/9**(211**)	9/9**(56**)	0.0**	1.0**
H_2O	-	-	-	-	0/20	5.2	3.6	0/14(9744)	0/14(8499)	4.3	5.8
Normals	-	3/3	4.8	4.4	-	-	0.0	4/5(152)	5/5(32)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

[g] Mean drinking water consumption in ml for the 7 day treatment.

[h] Ribavirin given bid x 5, 4 hr pre-virus inoculation. p.o. in sterile H_2O.

Conclusions: AVS3593 (LY 2531963), administered continuously in drinking water to PTV-infected mice, was moderately effective in this study. This regimen was recommended to us by USAMRIID personnel.

Table V-128. Expt. PtA301. Effect of Twice Daily s.c. Treatment With AVS3706 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.4-13.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3706	450	5/5	2.2	8/10**	7.5*
	225	5/5	2.8	6/10**	6.0
	112.5	5/5	3.1	2/10	5.1
	56.3	5/5	3.7	0/10	4.5
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Saline	-	-	-	1/20	4.6
Normals	-	5/5	3.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound, tiazofurin triacetate, was significantly inhibitory to PTV in this initial experiment. This experiment will be repeated to confirm these results.

Table V-129. Expt. PtA405. Effect of Twice Daily s.c. Treatment with AVS3706 on Punta Toro Virus Infections in Mice.
Animals: 12.9-14.1g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3706	600	5/5	2.5	10/10**	>21.0**	0.5**	3/10(425**)	2/10(407**)	3.1**	4.7**
	300	5/5	2.6	2/10*	6.1**	1.4	0/9(3723**)	0/9(1914**)	4.7	5.5**
	150	5/5	3.2	0/10	5.3**	1.6	0/10(5285*)	0/10(4696**)	4.0*	5.8*
	75	5/5	3.6	0/10	4.4	1.4	0/10(8570)	0/10(5550**)	5.2	6.3
Ribavirin	75	5/5	3.3	10/10**	>21.0**	0.3**	10/10**(83**)	10/10**(24**)	0.0**	2.2**
Saline	-	-	-	0/20	4.3	1.4	1/18(8942)	1/18(9799)	5.0	6.3
Normals	-	5/5	4.1	-	-	0.5	5/5(78)	5/5(56)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: In this expanded experiment, tiazofurin triacetate was highly active vs PTV using all infection parameters. The material was very well tolerated at all doses used in the study, indicating a potentially highly significant TI.

*P<0.05 **P<0.01

Table V-130. Expt. PtA219-222. Effect of Time of Single Treatment with AVS3925 on Punta Toro Virus Infections in Mice.

Animals: 11.7-14.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Methocel, DMSO, Tween 80.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3925	4 hr pre	200	0/5	-	1/10	1.0
		100	5/5	-0.4	7/10	6.0
		50	5/5	-0.6	5/10	4.8
		25	5/5	0.0	6/10	6.0
	4 hr post	200	0/5	-	0/10	1.8
		100	5/5	-0.4	6/10	6.0
		50	5/5	-0.6	8/10	6.5
		25	5/5	0.0	10/10*	>21.0**
	24 hr post	200	0/5	-	0/10	2.0
		100	5/5	-0.4	3/10	6.6
		50	5/5	-0.6	7/10	5.0
		25	5/5	0.0	5/10	4.8
	48 hr post	200	0/5	-	0/10	3.1
		100	5/5	-0.4	9/10	5.0
		50	5/5	-0.6	8/10	5.5
		25	5/5	0.0	3/10	5.4
Ribavirin		500	5/5	0.5	10/10*	>21.0**
DMSO/Tween 80		-	5/5	-0.7	-	-
Methocel 25%		-	-	-	12/20	5.6
Normals		-	5/5	0.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3925 is the duPont material A2222-1. In PtA 189, single i.p. treatments given 24 hr pre-virus inoculation were not effective vs PTV *in vivo*. In the present study. a single dosage administered 4 hr post-virus inoculation was effective. The compound was placed in solution by first adding it to 100% DMSO with 0.1 ml Tween 80, then diluting this material in 0.25% methocel for the lower concentrations used, per the manufacturer's directions. We had concern, however, that the DMSO-Tween 80 might be toxic to the animals, so a separate toxicity control was run using these substances only. While not lethally toxic, the animals appeared weakened from this inoculum, since the toxicity controls lost 0.7 g compared to a weight gain of 0.1 g during the same period in the normal controls. This suggests that the material should be tested further without use of the DMSO/Tween 80 initial vehicle.

Table V-131. Expt. PtA300. Effect of Once Only i.p. Treatment With AVS3925 on Punta Toro Virus Infections in Mice.

Animals: 12.8-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3925	25	5/5	2.1	1/10	4.4
	12.5	5/5	1.6	0/10	5.0
	6.25	5/5	2.0	1/10	4.4
	3.13	5/5	1.8	0/10	4.2
Ribavirin	350	5/5	1.7	8/10**	6.0
CMC	-	-	-	0/20	4.7
Normals	-	5/5	1.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound, duPont A2222-1, was tested previously after using a DMSO/Tween 80 diluent which we suspected was toxic to the mouse. The present experiment repeated the previous study, using lower dosages and standard CMC diluent. No activity was seen, but the material appeared well tolerated. This experiment will be repeated using higher doses.

Table V-132. Expt. PtA406. Effect of Once Daily i.p. Treatment With AVS3925 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, 36 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3925	25	5/5	1.9	0/10	6.2
	12.5	5/5	2.5	0/10	6.8
	6.25	4/5	2.3	0/10	7.1*
	3.13	5/5	2.8	1/10	6.4
Ribavirin	75	5/5	2.3	10/10**	>21.0**
CMC	-	-	-	1/20	6.4
Normals	-	5/5	3.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: DuPont A2222-1, used on a qd x 5 treatment schedule beginning 36 hr prior to virus inoculation, was not effective vs PTV in this experiment.

Table V-133. Expts. PtA418-420. Effect of Once Only i.p. Treatment With AVS3925 Administered at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.9-14.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3925	24 hr pre	200	5/5	-1.0	1/10	6.6
		100	5/5	0.4	1/10	6.2
		50	5/5	0.4	5/10*	6.4
		25	5/5	0.5	7/10**	6.3
	4 hr pre	200	5/5	-1.0	6/10*	5.3
		100	5/5	0.4	2/10	4.8
		50	5/5	0.4	1/10	4.1
		25	5/5	0.5	0/10	5.1
	4 hr post	200	5/5	-1.0	1/10	6.3
		100	5/5	0.4	0/10	5.1
		50	5/5	0.4	1/10	5.0
		25	5/5	0.5	0/10	4.4
Ribavirin		350	5/5	0.3	9/10**	5.0
CMC		-	-	-	3/20	5.8
Normals		-	5/5	1.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: DuPont A2222-1 was effective vs PTV when administered in single i.p. treatments beginning prior to virus inoculation.

Table V-134. Expt. PtA441. Effect of Every Other Day i.p. Treatments With AVS3925 on Punta Toro Virus Infections in Mice.

Animals: 11.4-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Three times, 24 hr pre- 24 hr post- & 72 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3925	40	5/5	2.9	0/10	5.1
	20	5/5	3.4	0/10	5.5
	10	5/5	4.2	1/10	5.6
	5	5/5	3.2	0/10	5.3
	2.5	5/5	3.4	1/10	5.2
CMC	-	-	-	0/20	5.3
Normals	-	5/5	2.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: DuPont A2222-1 was ineffective vs PTV when administered on an every other day treatment schedule.

Table V-135. Expt. PtA442. Effect of Twice Daily i.p. Treatment With AVS3925 on Punta Toro Virus Infections in Mice.

Animals: 11.2-12.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3925	40	5/5	3.3	0/10	4.9
	20	5/5	4.3	0/10	5.0
	10	5/5	3.1	0/10	5.0
	5	5/5	3.4	0/10	5.1
	2.5	5/5	3.1	0/10	4.9
Ribavirin[c]	75	5/5	2.9	9/10**	11.0
CMC	-	-	-	0/20	5.1
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin diluent is saline

*P<0.05 **P<0.01

Conclusions: DuPont A2222-1 was ineffective vs PTV when administered on a bid x 5 treatment schedule.

Table V-136. Expt. PtA223-226. Effect of Time of Single Treatment with AVS3926 on Punta Toro Virus Infections in Mice.

Animals: 11.7-14.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Methocel, DMSO, Tween 80.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3926	4 hr pre	200	0/5	-	0/10	1.0
		100	0/5	-0.7	2/10	1.1
		50	5/5	-0.7	3/10	6.0
		25	5/5	-0.3	4/10	5.0
	4 hr post	200	0/5	-	0/10	1.0
		100	0/5	-0.7	0/10	1.1
		50	5/5	-0.7	1/10	4.9
		25	5/5	-0.3	1/10	5.8
	24 hr post	200	0/5	-	0/10	2.2
		100	0/5	-0.7	1/10	3.0
		50	5/5	-0.7	8/10	6.5
		25	5/5	-0.3	6/10	6.5
	48 hr post	200	0/5	-	0/10	3.0
		100	0/5	-0.7	1/10	4.1
		50	5/5	-0.7	2/10	5.5
		25	5/5	-0.3	3/10	4.9
Ribavirin		500	5/5	0.5	10/10*	>21.0**
DMSO		-	5/5	-0.7	-	-
Methocel .25%		-	-	-	12/20	5.6
Normals		-	5/5	0.1	-	-

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3926 is the duPont material A2227-1, which was previously found ineffective vs PTV when administered i.p. in a single treatment 24 hr pre-virus inoculation. The present study was run to determine if varying the time of that single treatment relative to virus inoculation would affect the antiviral activity of the compound. No activity was seen at any time. However, the compound, like AVS3925, was placed in solution by first adding it to 100% DMSO with 0.1 ml Tween 80, then diluting the material in 0.25% methocel for the lower concentrations used, per the manufacturer's directions. We had concern, however, that the DMSO-Tween 80 might be toxic to the animals, so a separate toxicity control was run using these substances only. The animals appeared weakened by this inoculum, losing weight compared to untreated normal controls. We note that AVS3926 treatment was either lethally toxic or caused weight loss at all concentrations used. This effect may be due in part at least, to the DMSO/Tween 80 initial vehicle. The experiment should therefore be repeated using AVS3926 in a more suitable vehicle.

Table V-137. Expt. PtA421-422. Effect of Once Only i.p. Treatment With AVS3926 At 24 or 4 hr Pre-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.7-14.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 or 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3926	24 hr pre	100	5/5	-0.2	0/10	6.9*
		50	5/5	0.1	2/10	5.9
		25	5/5	0.2	5/10*	6.6
		12.5	5/5	0.4	2/10	6.0
	4 hr pre	100	5/5	-0.2	6/10*	5.3
		50	5/5	0.1	2/10	4.5
		25	5/5	0.2	7/10**	4.7
		12.5	5/5	0.4	2/10	6.1
Ribavirin		350	5/5	0.3	9/10**	5.0
CMC		-	-	-	3/20	5.8
Normals		-	5/5	1.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: DuPont A2227-1 was significantly active vs PTV in this experiment, especially when administered in a single i.p. injection 4 hr pre-virus inoculation.

Table V-138. Expt. PtA443. Effect of Twice Daily i.p. Treatment With AVS3926 on Punta Toro Virus Infections in Mice.

Animals: 11.4-12.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3926	40	5/5	3.2	0/10	5.0
	20	5/5	3.7	0/10	5.1
	10	5/5	2.6	0/10	5.2
	5	5/5	3.9	1/10	5.1
	2.5	5/5	3.4	0/10	5.0
Ribavirin	75	5/5	2.9	9/10**	11.0
CMC	-	-	-	0/20	5.1
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: DuPont A2227-1 was ineffective vs PTV when administered on a bid x 5 treatment schedule.

Table V-139. Expt. PtA227-230. Effect of Time of Single Treatment with AVS3927 on Punta Toro Virus Infections in Mice.

Animals: 11.7-14.1 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Methocel, DMSO, Tween 80.

Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3927	4 hr pre	200	4/5	-0.9	0/10	4.4
		100	4/4	1.0	3/10	5.7
		50	5/5	0.1	4/10	5.5
		25	5/5	-0.4	4/10	5.5
	4 hr post	200	4/5	-0.9	0/10	5.9
		100	4/4	1.0	3/10	5.9
		50	5/5	0.1	1/10	5.7
		25	5/5	-0.4	8/10	6.0
	24 hr post	200	4/5	-0.9	1/10	3.3
		100	4/4	1.0	7/10	5.3
		50	5/5	0.1	3/10	5.7
		25	5/5	-0.4	6/10	5.5
	48 hr post	200	4/5	-0.9	0/10	4.3
		100	4/4	1.0	1/10	5.3
		50	5/5	0.1	8/10	5.5
		25	5/5	-0.4	6/10	6.3
Ribavirin		500	5/5	0.5	10/10*	>21.0**
DMSO		-	5/5	-0.7	-	-
Methocel 25%		-	-	-	12/20	5.6
Normals		-	5/5	0.1	-	-

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3927 is the duPont material A754-1, which was previously found ineffective vs PTV when administered i.p. in a single treatment 24 hr pre-virus inoculation. The present study was run to determine if varying the time of that single treatment relative to virus inoculation would affect the antiviral activity of the compound. No activity was seen at any time. However, the compound, like AVS3925, was placed in solution by first adding it to 100% DMSO with 0.1 ml Tween 80, then diluting the material in 0.25% methocel for the lower concentrations used, per the manufacturer's directions. We had concern, however, that the DMSO-Tween 80 might be toxic to the animals, so a separate toxicity control was run using these substances only. The animals appeared weakened by this inoculum, losing weight compared to untreated normal controls. We note that AVS3926 treatment was either lethally toxic or caused weight loss at most concentrations used. This effect may be due in part at least, to the DMSO/Tween 80 initial vehicle. The experiment should therefore be repeated using AVS3927 in a more suitable vehicle.

Table V-140. Expt. PtA341. Effect of Once Daily i.p. Treatment With AVS3927 on Punta Toro Virus Infections in Mice (CMC Vehicle).

Animals: 9.3-10.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: Once daily x 5, 36 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3927	25	5/5	3.2	0/10	6.4
	12.5	5/5	2.9	0/9	6.7
	6.25	5/5	1.5	3/10	6.6
	3.13	5/5	2.9	1/10	6.3
Ribavirin[c]	75	5/5	2.7	10/10**	>21.0**
CMC	-	-	-	0/20	6.0
Normals	-	5/5	3.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered 4 hr pre-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This compound, Dupont 754-1, was previously inactive vs PTV but also poorly tolerated in the mouse, presumably due to the DMSO + Tween 80 vehicle used. In the present study, CMC was used as vehicle, and the material was well tolerated, but again was inactive. Higher dosages should be evaluated if adequate compound is available.

Table V-141. Expt. PtA411. Effect of Twice Daily i.p. Treatment With AVS3927 on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3927	25	5/5	3.2	5/10	7.4
	12.5	5/5	3.1	2/10	6.1
	6.25	4/5	2.8	4/10	5.3
	3.13	5/5	3.4	1/10	5.4
Ribavirin	75	5/5	2.2	10/10**	>21.0**
CMC	-	-	-	7/20	6.5
Normals	-	5/5	3.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: DuPont A754-1 was ineffective vs PTV when administered i.p. on a bid x 5 treatment schedule beginning 24 hr prior to virus inoculation.

Table V-142. Expt. PtA423-424. Effect of Once Only i.p. Treatment With AVS3927 At 24 or 4 hr Pre-Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.9-14.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 or 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3927	24 hr pre	200	5/5	0.0	1/10	5.7
		100	5/5	0.5	0/10	5.7
		50	5/5	0.4	2/10	6.3
		25	5/5	0.1	0/10	6.2
	4 hr pre	200	5/5	0.0	5/10*	6.8
		100	5/5	0.5	4/10	4.8
		50	5/5	0.4	3/10	5.4
		25	5/5	0.1	3/10	5.4
Ribavirin		350	5/5	0.3	9/10**	5.0
CMC		-	-	-	3/20	5.8
Normals		-	5/5	1.0	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: DuPont 754-1 was significantly effective vs PTV when administered i.p. in a single treatment 4 hr pre-virus inoculation.

Table V-143. Expt. PtA444. Effect of Twice Daily i.p. Treatment With AVS3927 on Punta Toro Virus Infections in Mice.

Animals: 11.1-12.7 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3927	40	5/5	2.9	0/10	5.0
	20	5/5	2.5	1/10	5.4
	10	5/5	3.7	0/10	5.0
	5	4/4	3.7	0/10	5.2
	2.5	5/5	3.3	0/10	5.4
Ribavirin[c]	75	5/5	2.9	9/10**	11.0
CMC	-	-	-	0/20	5.1
Normals	-	5/5	3.7	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin diluent is saline.

*P<0.05 **P<0.01

Conclusions: DuPont A754-1 was ineffective vs PTV when used on a bid x 5 treatment schedule. In this experiment, which essentially repeats PtA 411, a higher initial dose was used and a somewhat more potent virus inoculum was used.

Table V-144. Expt. PtA303. Effect of Once Daily i.p. Treatment With AVS3933 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.9-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3933	250	5/5	3.1	0/9	5.8
	125	5/5	2.7	1/10	5.9
	62.5	5/5	2.3	1/10	5.2
	31.3	5/5	2.2	1/10	5.4
Ribavirin	75	5/5	2.6	10/10**	>21.0**
Saline	-	-	-	1/20	4.6
Normals	-	5/5	1.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this initial experiment with Ge089, no activity was seen although the material was well tolerated. The experiment will be repeated using higher dosages, and also, since with a related material (Ge132, AVS3934) a bid x 7 treatment schedule was highly effective, this latter schedule will also be studied with this compound.

Table V-145. Expt. PtA218. Effect of Once Daily i.p. Treatment with AVS3934 on Punta Toro Virus Infections in Mice.

Animals: 12.0-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 0.4% CMC.

Treatment Schedule: Once daily x 7, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.

Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3934	300	5/5	-0.1	10/10**	>21.0**
	150	5/5	3.3	6/10	6.5
	75	5/5	2.5	5/10	6.2
	37.5	4/5	1.4	8/10	6.5
	18.8	5/5	3.7	7/10	7.0
Ribavirin	75	4/5	3.3	10/10**	>21.0**
CMC	-	-	-	13/20	6.1
Normals	-	5/5	4.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound is Ge132, which we found previously (PtA 192) to be inactive vs PTV *in vivo* when administered p.o. The present experiment was run to determine if i.p. treatment would render a more positive effect. Significant activity was seen at 300 mg/kg/day, which was approximately the MTD.

Table V-146. Expts. PtA367-368. Effect of Twice Daily i.p. Treatments with AVS3934 Begun at Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.2-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 7, beginning 24 or 48 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control			Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3934	24 hr pre	300	5/5	2.4	10/10**	>21.0**
		150	5/5	3.3	10/10**	>21.0**
		75	5/5	1.7	10/10**	>21.0**
		37.5	5/5	3.8	10/10**	>21.0**
	4 hr pre	300	5/5	2.4	10/10**	>21.0**
		150	5/5	3.3	10/10**	>21.0**
		75	5/5	1.7	10/10**	>21.0**
		37.5	5/5	3.8	10/10**	>21.0**
Ribavirin	4 hr pre	75	5/5	2.4	10/10**	>21.0**
Saline	4 hr pre	-	-	-	5/20	5.0
Normals		-	5/5	2.8	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: Ge 132 administered by a bid x 7 treatment schedule was highly active vs PTV whether given 24 hr pre or 4 hr pre-virus inoculation.

Table V-147. Expt. PtA387. Effect of Twice Daily i.p. Treatment with AVS3934 on Punta Toro Virus Infections in Mice.
Animals: 12.0-13.6g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3934	300	5/5	2.0	2/10	5.4**	1.8	0/10(1977)	0/10(2994)	4.8	5.7
	150	5/5	2.0	3/10	5.9**	2.1	1/9(4129)	1/9(3674)	4.8	5.7
	75	5/5	2.4	3/9	6.2**	2.4	0/10(5782)	0/10(5711)	5.1	6.0
	37.5	5/5	2.2	1/10	5.2**	1.8	0/10(4230)	0/10(3701)	5.2	5.8
	18.8	5/5	2.9	0/10	4.8	1.6	1/10(3721)	0/10(5095)	5.0	6.1
	9.4	5/5	2.2	1/10	6.1**	2.1	2/10(4558)	2/10(5598)	5.4	6.1
	4.7	5/5	2.5	0/10^	7.6**	2.4	0/10(6649)	0/10(7990)	4.7	5.4
Ribavirin	75	5/5	1.7	10/10**	>21.0**	0.7**	9/10**(133**)	10/10**(16**)	1.4**	0.6**
Saline	-	-	-	2/20	4.4	2.0	2/19(4217)	3/19(5034)	4.7	5.9
Normals	-	5/5	2.3	-	-	0.5	5/5(64)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^Two alive on day 17, day 18 all dead with no water in cage.

Conclusions: In previous experiments (PtA 367, 368), pretreatment with AVS3934 (Ge132) was highly effective in preventing death in PTV-infected mice. In the present study, the i.p. treatment beginning 4 hr pre-virus inoculation was only weakly effective, resulting in increases in mean survival time only.

*P<0.05 **P<0.01

Table V-148. Expt. PtA388. Effect of Twice Daily p.o. Treatment with AVS3934 on Punta Toro Virus Infections in Mice.

Animals: 11.8-13.4g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.
Treatment Schedule: Twice daily x 7, beginning 4 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3934	300^	5/5	3.0	1/10	4.6	0.7**	0/10(5766)	0/10(5524)	5.5	5.9
	150	5/5	3.2	1/10	4.3	0.6**	0/10(4144)	0/10(4234)	5.2	5.7
	75	5/5	3.4	1/10	5.7	1.1*	0/10(5404)	0/10(4919)	5.3	5.8
	37.5	5/5	3.5	3/10	5.7	2.1	0/9(9353)	0/9(8983)	5.2	6.2
	18.8	5/5	2.9	4/10*	5.3	1.2*	1/9(4849)	1/9(3935)	5.2	6.1
	9.4	5/5	3.3	2/10	4.4	1.5	0/10(7230)	0/10(6470)	4.4	5.4
	4.7	5/5	2.4	1/10	5.2	1.5	0/10(8390)	0/10(8660)	5.2	5.6
Ribavirin	75	5/5	3.1	10/10**	>21.0**	0.1**	8/10**(161**)	8/10**(73**)	0.9**	2.3**
H_2O	-	-	-	1/20	4.2	2.0	0/18(4088)	0/18(4020)	4.6	5.4
Normals	-	5/5	2.3	-	-	0.5	5/5(64)	5/5(28)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^At this dose in both toxicity and infected, treated, there was a blue-green precipitate (oxidation) between the feeding tube and the syringe outside.

Conclusions: A previous experiment (PtA 368) with Ge132 showed i.p. therapy beginning 4 hr pre-virus inoculation was highly effective vs PTV infections. This experiment was run to determine if oral therapy using the same schedule would also be effective. The treatment resulted in reduction of liver scores only, although significant increase in survivors was seen at the 18.8 mg/kg/day dose only.

*P<0.05 **P<0.01

Table V-149. Expt. PtA485. Effect of Twice Daily i.p. Treatment with AVS3934 on Punta Toro Virus Infections in Mice.
Animals: 10.2-13.1 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily x 7, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3934	600	5/5	4.4	0/10	6.1	2.5	1/10(3616)	0/10(3424)	3.8	4.0
	300	5/5	4.6	5/10	7.0	3.1	0/10(3440)	0/10(3292)	4.6	4.7
	150	5/5	3.6	6/10	6.3	2.7	0/10(2627)	0/10(2712)	3.8	4.0
	75	5/5	4.4	2/10	6.9	3.3	0/10(2576)	0/10(2417)	4.6	4.6
	37.5	5/5	3.4	0/10	7.0	2.5	0/10(3615)	1/10(3233)	4.6	4.2
	18.8	5/5	3.6	0/10	6.0	2.8	0/9(3102)	0/9(3444)	4.8	4.4
Ribavirin	75	5/5	3.9	10/10**	>21.0**	0.3**	10/10**(91**)	10/10**(24**)	0.8**	1.5**
Saline	-	-	-	9/20	6.1	2.4	1/17(2816)	1/17(2800)	4.3	4.1
Normals	-	5/5	6.0	-	-	0.2	5/5(114)	5/5(23)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Previous studies with AVS3834 (Ge 132, Germanium) indicated i.p. treatment twice daily for 7 days beginning 24 hr pre-virus inoculation was highly active vs PTV. This activity could not be confirmed in the present experiment. All previous experiments (PtA 192, 218, 367, 368, 387, 388) used a material that was supplied in powder form. The present study used a liquid supplied to us later, suggesting the material may be altered in the liquid preparation.

*P<0.05 **P<0.01

Table V-150. Expt. PtA486. Effect of Twice Daily p.o. Treatment with AVS3934 on Punta Toro Virus Infections in Mice.
Animals: 11.5-12.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.
Treatment Schedule: Twice daily x 7, beginning 24 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3934	600	5/5	3.9	0/10	5.8	2.4	0/9(6519)	0/9(5702)	5.2	5.5
	300	5/5	2.6	0/10	7.0	2.6	0/10(7401)	1/10(6300)	4.9	5.2
	150	5/5	4.2	3/10	6.4	2.8	0/10(8820)	0/10(8265)	5.7	5.1
	75	5/5	4.4	5/10*	6.4	2.3**	1/10(4333)	1/10(3977)	3.3	5.6
	37.5	5/5	3.9	0/10	6.6	3.0	0/10(7720)	0/10(8186)	5.4	5.5
	18.8	5/5	5.1	1/10	5.6	2.6	3/10(2413**)	3/10(2259**)	3.8	3.8
Ribavirin	75	5/5	4.6	10/10**	>21.0**	0.5**	10/10**(132**)	10/10**(33**)	0.9**	2.0*
H_2O	-	-	-	3/20	6.8	3.3	4/20(6490)	4/20(5630)	4.2	4.6
Normals	-	5/5	6.0	-	-	0.2	5/5(114)	5/5(23)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: Previous studies with AVS3934 (Ge 132, Germanium) indicated that i.p. treatment using this regimen to be effective vs PTV infections, but p.o. therapy by other regimens was not efficacious. As seen in PtA 485 using a new lot of AVS3934 in liquid form, no activity was seen via i.p. therapy. The present study used p.o. therapy with the liquid preparation, with activity seen at a mid-ranged dose only.

*P<0.05 **P<0.01

Table V-151. Expt. PtA487. Effect of Twice Daily p.o. Treatment with AVS3934 on Punta Toro Virus Infections in Mice.
Animals: 11.5-12.7 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.
Treatment Schedule: Twice daily x 7, beginning 48 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS3934	600	5/5	3.9	8/10**	7.5	2.7	2/10(4374)	2/10(3369)	4.4	4.0
	300	5/5	2.6	0/10	7.1	3.2	0/10(6887)	0/10(5248)	3.4	5.4
	150	5/5	4.2	2/10	7.3	2.0**	0/9(8084)	0/9(6736)	5.0	5.8
	75	5/5	4.4	3/10	6.9	1.1**	4/9(2113**)	6/9*(1617**)	2.0**	1.8**
	37.5	5/5	3.9	1/10	7.1	2.5	0/9(9351)	0/9(8192)	5.0	5.9
	18.8	5/5	5.1	3/10	7.3	2.5	0/10(11,118)	0/10(9470)	4.8	5.5
Ribavirin	75	5/5	4.6	10/10**	>21.0**	0.5**	10/10**(132**)	10/10**(33**)	0.9**	2.0**
H_2O	-	-	-	3/20	6.8	3.3	4/20(6490)	4/20(5630)	4.2	4.6
Normals	-	5/5	6.0	-	-	0.2	5/5(114)	5/5(23)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: In this study, AVS3934 (Ge 132, Germanium)) was moderately effective when administered orally beginning 48 hr prior to virus inoculation. This material was a new lot of AVS3934 supplied in liquid form.

*P<0.05 **P<0.01

Table V-152. Expt. PtA515-517. Effect of Once Only i.p. Treatment With AVS3934 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 13.0-13.6 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3934	24 hr pre	300	5/5	-0.1	0/10	6.0
		150	5/5	0.1	0/10	5.8
		75	5/5	0.1	0/10	5.8
		37.5	5/5	0.0	0/10	6.1
		18.8	5/5	0.2	2/10	6.0
	4 hr post	300	5/5	-0.1	0/10	5.5
		150	5/5	0.1	2/10	5.5
		75	5/5	0.1	0/10	5.2
		37.5	5/5	0.0	1/10	4.8
		18.8	5/5	0.2	0/10	4.4
	24 hr post	300	5/5	-0.1	0/10	4.7
		150	5/5	0.1	2/10	5.0
		75	5/5	0.1	0/10	5.1
		37.5	5/5	0.0	4/10	5.0
		18.8	5/5	0.2	0/10	5.2
Ribavirin		350	5/5	0.0	10/10**	>21.0**
Saline		-	-	-	7/20	5.2
Normals		-	5/5	0.4	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS3932 (Ge 132, Germanium) was ineffective vs PTV when used in a single i.p. injection.

Table V-153. Expt. PtA196. Effect of AVS3960 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 9.9-11.5 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Twice daily x 7, beginning 36 hr pre-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3960	800	5/5	1.6	-	-
	400	5/5	3.3	-	-
	200	5/5	3.0	-	-
	100	5/5	3.5	0/10	5.6
	50	5/5	3.9	0/10	5.1
	25	5/5	3.8	0/10	5.3
	12.5	5/5	4.2	0/10	5.3
	6.3	5/5	3.0	0/10	5.6
Ribavirin	75	5/5	2.6	10/10**	>21.0**
H_2O	-	-	-	1/20	5.2
Normals	-	5/5	4.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This is the initial test run with DMG vs PTV *in vivo*. The dosage schedule was as recommended by the manufacturer. No activity was seen, although the compound was not tested at the higher concentrations, which were included in an effort to define the MTD. The compound was well tolerated at all dosages used, although the animals gained less weight at the highest dosage used, suggesting the MTD was being approached. With this lack of toxicity, more studies need to be run with this compound.

Table V-154. Expt. PtA197. Effect of AVS3960 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 9.6-11.3 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 7, beginning 36 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3960	800	5/5	3.3	-	-
	400	5/5	3.9	-	-
	200	5/5	4.0	-	-
	100	5/5	4.4	0/10	5.5
	50	5/5	3.7	1/10	5.4
	25	5/5	3.3	0/10	5.6
	12.5	5/5	3.0	0/10	5.1
	6.3	5/5	3.2	0/10	5.1
Ribavirin	75	5/5	2.8	9/10**	11.0
Saline	-	-	-	0/20	5.2
Normals	-	5/5	4.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This test was run in parallel with PtA 196 using DMG. The treatment regimen was as recommended by the manufacturer, with the higher dosages run to determine toxicity only. The material was well tolerated at all dosages employed, suggesting a need for further work with the compound.

Table V-155. Expt. PtA349. Effect of Twice Daily s.c. Treatment With AVS3960 on Punta Toro Virus Infections in Mice.

Animals: 10.2-11.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, beginning 24 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS3960	900	5/5	2.1	0/10	5.1
	450	5/5	2.2	0/10	4.9
	225	5/5	2.2	0/10	4.9
	112.5	5/5	3.0	0/10	4.6
Ribavirin[c]	75	5/5	2.7	10/10**	>21.0**
Saline	-	-	-	0/20	4.9
Normals	-	5/5	3.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Ribavirin administered 4 hr pre-virus inoculation.

*P<0.05 **P<0.01

Conclusions: DMG was ineffective vs PTV in this study, which extends a previous experiment (PTA 197) to include a higher dosage level of the compound. The material was still well tolerated, indicating possibly additional work should be done with it.

Table V-156. Expt. PtA433. Effect of Once Daily s.c. Treatment With AVS4113 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 11.8-14.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4113	12	5/5	2.0	0/10	4.4
	6	5/5	3.4	0/10	5.8
	3	5/5	1.9	1/10	6.0
	1.5	5/5	3.5	0/10	6.3
	0.75	5/5	2.4	0/10	6.3*
Ribavirin	75	5/5	2.0	10/10**	>21.0**
Saline	-	-	-	1/20	5.3
Normals	-	5/5	2.6	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: In this initial experiment with pseudolycorine•HCl, no anti-PTV activity was seen. The compound was well tolerated at all doses used, however, suggesting higher doses should be examined.

Table V-157. Expts. PtA463-465. Effect of Single i.p. Treatment With AVS4282 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4282	24 hr pre	200	0/5	-	0/10	1.0
		100	0/5	-	0/10	1.1
		50	0/5	-	0/10	1.0
		25	0/5	-	0/10	1.6
		12.5	0/5	-	0/10	2.0
	4 hr post	50	1/5	-2.8	0/10	1.7
		25	0/5	-0.6	0/10	1.9
		12.5	0/5	-1.6	0/10	2.7
		6.25	0/5	-0.2	0/10	2.7
		3.125	0/5	-1.7	0/10	3.8
	24 hr post	50	1/5	-2.8	0/10	2.9
		25	0/5	-0.6	0/10	2.3
		12.5	0/5	-1.6	0/10	3.0
		6.25	0/5	-0.2	0/10	3.3
		3.125	0/5	-1.7	0/10	4.2**
Ribavirin		350	5/5	-0.2	6/10**	7.3**
Saline		-	-	-	0/20	3.6
Normals		-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4284 (AM-5), at these concentrations, was very toxic, killing almost all mice before infection with PTV.

Table V-158. Expt. PtA494-496. Effect of Once Only i.p. Treatment With AVS4282 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 13.0-15.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4282	24 hr pre	0.8	5/5	-1.0	1/10	6.1
		0.4	5/5	-0.8	0/10	6.5
		0.2	5/5	-0.3	2/10	6.4
		0.1	5/5	-0.5	2/10	6.5
		0.05	5/5	-0.1	0/10	6.7*
		0.025	5/5	-0.2	3/10	6.3
	4 hr post	0.8	5/5	-1.0	0/10	5.4
		0.4	5/5	-0.8	6/10*	5.8
		0.2	5/5	-0.3	4/10	5.8
		0.1	5/5	-0.5	1/10	5.6
		0.05	5/5	-0.1	4/10	6.0
		0.025	5/5	-0.2	5/10*	7.6**
	24 hr post	0.8	5/5	-1.0	10/10**	>21.0**
		0.4	5/5	-0.8	9/10**	7.0
		0.2	5/5	-0.3	10/10**	>21.0**
		0.1	5/5	-0.5	8/10**	5.5
		0.05	5/5	-0.1	8/10**	5.5
		0.025	5/5	-0.2	5/10*	5.4
Ribavirin		350	5/5	-0.8	9/10*	10.0
Saline		-	-	-	3/20	5.6
Normals		-	5/5	-0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4282 (AM-5), an apparent immunomodulator, was highly active vs PTV in this experiment, especially when administered after virus inoculation.

Table V-159. Expts. PtA466-468. Effect of Single I.p. Treatment With AVS4283 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4283	24 hr pre	200	5/5	-1.0	0/10	5.1**
		100	5/5	-0.6	1/10	5.6**
		50	5/5	-0.4	0/10	5.2**
		25	5/5	-0.3	2/10*	5.1**
		12.5	5/5	-0.1	1/10	5.1**
	4 hr post	200	5/5	-1.0	0/10	4.2**
		100	5/5	-0.6	0/10	3.9
		50	5/5	-0.4	0/10	3.8
		25	5/5	-0.3	2/10*	4.0
		12.5	5/5	-0.1	0/10	3.5
	24 hr post	200	5/5	-1.0	0/10	3.6
		100	5/5	-0.6	2/10*	5.6**
		50	5/5	-0.4	5/10**	5.0**
		25	5/5	-0.3	0/10	3.8
		12.5	5/5	-0.1	0/10	4.3**
Ribavirin		350	5/5	-0.2	6/10**	7.3**
Saline		-	-	-	0/20	3.6
Normals		-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4283 (AM-6), an apparent immunomodulator, was effective vs PTV at all treatment times in this study.

Table V-160. Expts. PtA469-471. Effect of Single i.p. Treatment With AVS4284 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.3-12.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4284	24 hr pre	180	5/5	-0.3	0/10	5.4**
		90	5/5	0.2	0/10	5.1**
		45	5/5	0.2	0/10	5.2**
		22.5	5/5	0.1	0/10	4.9**
		11.25	5/5	0.3	0/10	5.0**
	4 hr post	180	5/5	-0.3	0/10	3.5
		90	5/5	0.2	0/10	3.7
		45	5/5	0.2	0/10	3.6
		22.5	5/5	0.1	0/10	3.1
		11.25	5/5	0.3	0/10	3.8
	24 hr post	180	5/5	-0.3	0/10	4.7**
		90	5/5	0.2	0/10	5.1**
		45	5/5	0.2	0/10	3.9
		22.5	5/5	0.1	0/10	4.6**
		11.25	5/5	0.3	0/10	3.7
Ribavirin		350	5/5	-0.2	6/10**	7.3**
Saline		-	-	-	0/20	3.6
Normals		-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4284 (AM-7), an apparent immunomodulator, was moderately active vs PTV in these experiments.

Table V-161. Expt. PtA472. Effect of Single i.p. Treatment With AVS4285 on Punta Toro Virus Infections in Mice.

Animals: 10.6-12.2 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4285	100	5/5	-0.3	0/10	5.5
	50	5/5	0.2	1/10	5.8
	25	5/5	0.2	1/10	4.9
	12.5	5/5	0.1	1/10	4.8
	6.25	5/5	-0.3	0/10	4.8
Ribavirin	350	5/5	-0.2	6/10**	7.3**
Saline	-	-	-	1/20	5.5
Normals	-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4285 (AM-8), an apparent immunomodulator, was ineffective vs PTV in this experiment.

Table V-162. Expt. PtA488-490. Effect of Once Only i.p. Treatment With AVS4286 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.8-15.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4286	24 hr pre	200	5/5	-	8/10*	8.0**
		100	5/5	-	10/10**	>21.0**
		50	5/5	-	10/10**	>21.0**
		25	5/5	-	7/10	6.0
		12.5	5/5	-	1/10	7.0**
	4 hr post	200	5/5	-	9/10**	10.0
		100	5/5	-	10/10**	>21.0**
		50	5/5	-	10/10**	>21.0**
		25	5/5	-	10/10**	>21.0**
		12.5	5/5	-	5/10	5.0
	24 hr post	200	5/5	-	10/10**	>21.0**
		100	5/5	-	10/10**	>21.0**
		50	5/5	-	2/10	10.6**
		25	5/5	-	10/10**	>21.0**
		12.5	5/5	-	9/10**	17.0
Ribavirin		350	5/5	-	10/10**	>21.0**
Saline		-	-	-	8/20	5.2
Normals		-	5/5	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4286 (P-136), an apparent immunomodulator, was highly active vs PTV in this study.

Table V-163. Expt. PtA478-480. Effect of Once Only i.p. Treatment With AVS4287 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 11.3-14.9 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4287	24 hr pre	200	5/5	-0.7	2/10	7.0**
		100	5/5	-0.7	3/10	7.1**
		50	5/5	-0.3	0/10	7.4**
		25	5/5	-0.3	2/10	6.0**
		12.5	5/5	-0.1	1/10	6.0**
	4 hr post	200	5/5	-0.7	1/10	5.8**
		100	5/5	-0.7	5/10	7.6**
		50	5/5	-0.3	6/10*	7.3**
		25	5/5	-0.3	9/10**	5.0
		12.5	5/5	-0.1	0/10	5.3
	24 hr post	200	5/5	-0.7	0/10	5.0
		100	5/5	-0.7	8/10**	5.0
		50	5/5	-0.3	7/10**	5.7
		25	5/5	-0.3	10/10**	>21.0**
		12.5	5/5	-0.1	10/10**	>21.0**
Ribavirin		350	5/5	0.4	9/10**	7.0
Saline		-	-	-	4/20	4.8
Normals		-	5/5	0.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4287 (P-117), an apparent immunomodulator, was highly active vs PTV in this study, especially when given 24 hr after virus inoculation.

Table V-164. Expt. PtA504. Effect of Single i.p. Treatment with AVS4287 on Punta Toro Virus Infections in Mice.

Animals: 11.8-14.0 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS4287	50	5/5	-1.2	10/10**	>21.0**	0.4**	10/10**(106**)	10/10**(32**)	0.0**	0.2**
	25	5/5	-0.8	10/10**	>21.0**	1.0**	7/10**(233**)	8/10**(91**)	1.2**	0.3**
	12.5	5/5	-0.2	10/10**	>21.0**	0.8**	6/10**(271**)	6/10**(139**)	0.5**	0.2**
	6.25	5/5	-0.3	10/10**	>21.0**	1.1**	1/10(384**)	1/10(291**)	0.3**	0.4**
	3.13	5/5	0.3	9/10**	4.0	0.9**	0/10(797**)	0/10(1002**)	0.3**	0.7**
	1.56	5/5	0.3	9/10**	4.0	0.5**	0/10(1666)	0/10(1569**)	1.4**	1.9**
	0.78	5/5	0.2	7/10	5.3	1.2**	1/10(1775**)	0/10(2929**)	2.4**	3.0**
Ribavirin	350	5/5	0.0	10/10**	>21.0**	0.5**	8/10**(165**)	9/10**(79**)	1.6**	4.3
Saline	-	-	-	7/20	5.2	3.1	0/20(7571)	0/20(7772)	5.1	4.8
Normals	-	5/5	0.4	-	-	0.0	5/5(94)	5/5(22)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: AVS4287 (P-117) was highly active vs PTV in this confirming experiment. Significant activity was seen at 6 dosages, indicting a wide therapeutic index for this material.

*P<0.05 **P<0.01

Table V-165. Expt. PtA482-484. Effect of Once Only i.p. Treatment With AVS4593 At Varying Times Relative to Virus Inoculation on Punta Toro Virus Infections in Mice.

Animals: 12.8-15.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, at varying times relative to virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

			Tox. Control		Infected, Treated	
Compound	Time of Treatment	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4593	24 hr pre	200	5/5	0.1	1/10	5.8**
		100	5/5	0.1	1/10	5.4**
		50	5/5	0.0	2/10	5.8**
		25	5/5	-0.2	0/10	5.3**
		12.5	5/5	-0.3	0/10	5.2**
	4 hr pre	200	5/5	0.1	0/10	4.2
		100	5/5	0.1	0/10	5.1
		50	5/5	0.0	3/10	5.1
		25	5/5	-0.2	2/10	5.8**
		12.5	5/5	-0.3	1/10	5.9**
	24 hr post	200	5/5	0.1	5/10*	6.4
		100	5/5	0.1	8/10**	5.5
		50	5/5	0.0	5/10*	5.0
		25	5/5	-0.2	10/10**	>21.0**
		12.5	5/5	-0.3	4/10	5.8**
Ribavirin		350	5/5	0.2	8/10**	8.5
Saline		-	-	-	2/20	4.4
Normals		-	5/5	0.5	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS4593 (P-188), an apparent immunomodulator, was highly active vs PTV in this study, especially when given after virus exposure.

Table V-166. Expt. PtA412. Effect of Twice Daily s.c. Treatment With AVS4616 on Punta Toro Virus Infections in Mice (Initial Test).

Animals: 12.6-13.8 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Twice daily x 5, 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS4616	150	5/5	3.5	2/10	5.3
	75	5/5	3.1	3/10	4.6
	37.5	4/5	2.6	0/10	5.1
	18.8	5/5	3.0	2/10	5.9
	9.4	5/5	2.6	6/10	5.8
Ribavirin	75	5/5	2.2	10/10**	>21.0**
Saline	-	-	-	9/20	5.0
Normals	-	5/5	3.2	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This compound, noxymethyl pennicillinic acid, was ineffective vs PTV in this experiment.

VI. EFFECT OF AVS COMPOUNDS ON INTRACEREBRAL INFECTIONS IN MICE INDUCED BY THE BALLIET STRAIN OF PUNTA TORO VIRUS

Introduction

It has been stressed from the inception of this project that the PTV infection in mice is being used as a model for Rift Valley fever and sandfly fever infections in man. A late and often fatal form of Rift Valley fever involves encephalitis, and patients with sandfly fever also develop certain symptoms suggestive of central nervous system (CNS) infection. We therefore felt it was important to determine if AVS compounds active against the hepatotropic Adames PTV infection would also have an effect on an encephalitic disease induced in mice by the neurotropic (Balliet) strain of PTV. As described earlier, our protocol for in vivo evaluations of anti-PTV compounds includes follow-up testing of PTV-inhibitory compounds against the CNS disease in mice. The results of these follow-up investigations are described in this section. Studies with i.v.-administered compounds are described in a separate section (XIII).

Materials and Methods

Virus: The Balliet strain of PTV as described in Sections I and III of our Annual Report No. 1 was used. A mouse brain-prepared virus pool was used in the present studies. The virus, suspended in Pucks balanced salt solution (PBSS) was used at dilutions of 10^{-3} or 10^{-4} (10 and 1LD50), coinciding with 10^4 and 10^3 Vero cell CCID50 of virus. The latter dose was used in most studies in an attempt to increase the sensitivity of the test.

Animals: Four week-old female Balb/c and Swiss Webster mice were obtained from Simonsen Laboratories. The animals were quarantined 48 hr prior to use and were maintained on standard mouse chow and water *ad libitum*.

Compounds: The following 13 AVS compounds were evaluated during this contract period: AVS111, 206, 272, 1761, 1767, 2149, 2776, 2777, 2880, 2933, 3588, 3589, and 3706. All were provided by Technassociates, Inc.

Experiment Design: Ether-anesthetized mice were infected by inoculating 0.05 ml of PTV i.c. into the right hemisphere of the brain. Twenty infected mice were used with each drug level, with 5 infected mice used as virus controls which received drug diluent only. Treatment and schedule varied depending upon the compound being evaluated, with those regimens considered highly effective against the hepatotropic virus infection selected for treatment of this CNS disease. Five toxicity control mice were used at each drug dose level, and 10 mice were used as normal controls. The latter two groups of controls were weighed before and after treatment as described in Section V. On infection day 6, one-half (one or two pre-designated cages) of each group of infected animals were killed and their brains removed. Ten percent homogenates of each brain were diluted through a series of 10-fold dilutions and each was assayed for virus using CPE production in triplicate cups of LLC-MK_2 cells. The remaining animals were observed daily for death through infection day 21, which was the termination of the experiment.

Increases in survivor number were evaluated using chi square analysis with Yate's correction. Increases in mean survival time and decreases in mean brain virus titers were analyzed using *t* test.

Results and Discussion

The results with each AVS compound tested against this CNS infection are shown in Tables VI-1 to 15. The following summarizes the activity of each. See also the conclusions indicated on each table.

AVS111 (Tiazofurin) (Table VI-1): No activity seen.

AVS206 (Ribavirin carboxamidine, ribamidine) (Table VI-2): Moderately effective vs the i.n.-instilled virus infection as seen by reduced virus titers in the brain at the highest dosage used (600 mg/kg/day). This dose is only approximately 1/4 the MTD, suggesting this material may have greater efficacy used at higher dosages. We previously found AVS206 to be moderately effective also against the i.c.-administered PTV.

AVS272 (3-Deazaguanine) (Table VI-3): No activity seen.

AVS1761 (Poly IC•LC) (Table VI-4): No activity seen.

AVS1767 (AM-3) (Table VI-5): No activity seen.

AVS2149 (Ampligen, poly1•polyC12U) (Table VI-6): We previously found this material to be significantly effective vs the i.c.-administered virus. Against the i.n.-administered PTV, however, no activity was seen. The data were masked to a degree, however, by low numbers of virus control animals dying and low amounts of virus recovered from the brains.

AVS2776 (Bropirimine, ABPP) (Table VI-7): Moderate activity was seen vs the i.n.-administered virus infection, manifested only as significant reductions in brain virus titers at two nontoxic dosage levels.

AVS2777 (AIPP) (Table VI-8): No activity seen by this derivative of AVS2776.

AVS2880 (Oxamisole) (Tables VI-9-11): Sporadic activity seen as decreased brain virus titers or increased mean survival times were seen in two experiments with this compound. A third experiment, run to confirm the initial activity seen by qd x 3 treatment, found no positive activity. We will investigate this material further.

AVS2933 (CPG 1935A) (Table VI-12): No activity seen.

AVS3588 (Metafluora derivative of ABPP) (Table VI-13): Moderate activity was seen at the low and high dosages used of this compound, indicated by decreased brain virus titers.

AVS3589 (5-Chloro-2,3-difluorophenyl derivative of ABPP) (Table VI-14): Moderate activity was seen at the high dose used; this was evidenced by significantly decreased brain virus titers.

AVS3706 (Tiazofurin triacetate) (Table VI-15): No activity seen.

Conclusions

Thirteen AVS compounds were evaluated against the CNS infection induced by the Balliet strain of PTV. Compounds AVS206, 2149, 2776, 2880, 3588, and 3589 were moderately effective, primarily as evidenced by reduction in recoverable brain virus titers.

Table VI-1. Expt. PtA182. Effect of AVS111 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 11.9 - 13.3 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily X 5, beginning 24 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Mean Brain Virus[c]
AVS111	500	5/5	1.0	1/9	10.4	5.7
	250	5/5	1.5	2/9	10.3	5.9
	125	5/5	2.2	1/9	10.1	5.9
	62.5	5/5	2.1	2/10	10.5	6.0
Saline	-	-	-	2/18	10.4	5.9
Normals	-	5/5	3.1	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Virus assayed on day 6 post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This is tiazofurin, which was previously found active vs Adames PTV infection using this treatment regimen. No activity was seen vs the i.c.-inoculated Balliet virus, however.

Table VI-2. Expt. PtA363. Effect of AVS206 on Intranasally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 11.6 - 13.6 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.n. administered.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS206	600	5/5	2.3	4/8	14.3	0.0**
	300	5/5	2.2	7/9	15.5	0.4
	150	5/5	2.6	9/10	16.3	0.7
	75	5/5	2.6	6/8	20.0	0.4
CMC	-	-	-	11/18	14.3	0.7
Normals	-	5/5	3.2	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: This experiment with the carboxamidine derivative of ribavirin used against the i.n.-administered virus infection essentially repeats an earlier study in which the virus was injected i.c. Activity was seen again as decreased brain virus titers.

Table VI-3. Expt. PtA343. Effect of AVS272 on Intracerebrally Injected Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 15.0 - 16.9 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS272	200	5/5	-1.1	0/10	7.9	6.4
	100	5/5	0.7	0/8	7.5	6.1
	50	5/5	0.9	0/9	8.8	6.0
	25	5/5	1.8	0/9	8.9	6.1
CMC	-	-	-	0/20	8.6	6.5
Normals	-	5/5	2.7	-	-	1.7

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: 3-Deazaguanine was highly active vs Adames PTV when administered s.c. by this qd x 5 treatment schedule. Against the i.c.-inoculated virus, however, no activity was seen.

Table VI-4. Expt. PtA361. Effect of AVS1761 on Intracerebrally Injected Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 13.1 - 17.2 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS1761	0.5	3/3	2.3	2/10	9.1	5.0
	0.25	3/3	2.0	1/10	8.3	5.0
	0.13	3/3	0.5	2/10	9.9	5.3
	0.06	3/3	1.2	3/9	10.5	4.9
Saline	-	-	-	3/20	9.9	5.9
Normals	-	3/3	2.2	-	-	1.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: Poly IC•LC was ineffective against the Balliet strain PTV in this experiment. The treatment regimen used was highly effective with this compound used against the Adames PTV infection.

Table VI-5. Expt. PtA168. Effect of AVS1767 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 11.9 - 13.3 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Twice daily X 5, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Mean Brain Virus[c]
AVS1767	500	4/5	-2.1	0/9	8.9	7.0
	250	5/5	1.3	0/10	9.3	6.0
	125	5/5	2.7	1/9	9.6	6.9
	62.5	5/5	1.8	0/10	9.0	6.8
Saline	-	-	-	1/18	9.8	6.3
Normals	-	5/5	3.0	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Virus assayed on day 6 post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This compound is AM-3, which was previously found to be active vs Adames PTV infections using this treatment regimen. No activity was seen vs the i.c. inoculated Balliet virus, however.

Table VI-6. Expt. PtA362. Effect of AVS2149 on Intranasally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 11.6 - 13.6 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.n. administered.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily x 5, beginning 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS2149	5	5/5	2.0	9/10	14.0	0.0
	2.5	5/5	1.4	8/10	17.0	0.0
	1.25	5/5	2.2	9/10	13.0	0.3
	0.63	5/5	3.4	6/9	15.0	0.0
Saline	-	-	-	15/20	15.2	0.4
Normals	-	5/5	3.2	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS2149 (Ampligen) was significantly effective against the i.c.-injected PTV. In the present experiment, altered only by i.n. administration, no statistically significant effects were seen, but these were masked to a degree by the low numbers of control mice dying and the low amount of virus recovered from the brains of the saline controls.

Table VI-7. Expt. PtA364. Effect of AVS2776 on Intranasally Administered Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 11.6 - 13.6 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.n. administered.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS2776	400	5/5	-0.2	6/8	19.5	0.3
	200	5/5	0.3	7/8	16.0	0.0**
	100	5/5	0.5	6/10	14.5	0.0**
	50	5/5	0.5	6/8	15.0	0.7
CMC	-	-	-	11/18	14.3	0.7
Normals	-	5/5	0.8	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: ABPP was moderately effective vs i.n.-administered Balliet PTV in this experiments. The erratic totals are a result of mice dying during the infection process, which involves anesthetizing the mice with ether.

Table VI-8. Expt. PtA174. Effect of AVS2777 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 12.9 - 14.1 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily X 3, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Mean Brain Virus[c]
AVS2777	300	5/5	0.3	0/9	10.6	6.4
	150	5/5	1.7	0/9	9.3	6.0
	75	5/5	2.0	2/10	8.5	6.5
	37.5	5/5	1.9	0/8	10.8	6.2
CMC	-	-	-	2/19	10.2	6.2
Normals	-	4/5	2.8	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Virus assayed on day 6 post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This is AIPP, which was previously found to be active vs Adames PTV by this treatment regimen. No activity was seen vs the i.c.-inoculated Balliet virus, however.

Table VI-9. Expt. PtA183. Effect of AVS2880 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 12.1 - 14.3 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Once daily X 3, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Mean Brain Virus[c]
AVS2880	25	5/5	1.1	1/10	10.2	5.8
	12.5	5/5	1.1	1/10	10.4	6.0
	6.25	5/5	1.7	1/10	10.2	5.5
	3.13	5/5	1.9	0/9	10.3	6.0
	1.55		1.6	0/9	12.0*	4.3**
Saline	-	-	-	2/18	10.4	5.9
Normals	-	5/5	3.1[d]	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Virus assayed on day 6 post-virus inoculation.

[d]Weight 3 days later than others.

*P<0.05 **P<0.01

Conclusions: This is oxamisole, which has previously been shown to have positive but erratic activity vs the Adames PTV by this treatment regimen. The compound, which is an immune modulator, exerted a positive effect at the lowest dosage used as seen by significantly increased MST and decreased mean brain virus titer.

Table VI-10. Expt. PtA184. Effect of AVS2880 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 12.1 - 14.3 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Once only, 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Mean Brain Virus[c]
AVS2880	50	5/5	-0.1	1/10	10.8	4.2**
	25	5/5	0.1	2/10	11.8*	5.6
	12.5	5/5	0.3	1/9	10.8	5.7
	6.25	5/5	0.7	2/10	11.5	5.3
	3.13		-0.3	0/10	9.3	6.0
Saline	-	-	-	2/18	10.4	5.9
Normals	-	5/5	0.2	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Virus assayed on day 6 post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This is oxamisole, which has previously been shown to have positive but erratic activity vs the Adames PTV by this treatment regimen. The compound, which is an immune modulator, exerted a positive effect at the 50 and 25 mg/kg dosages, the former as seen by decreased brain virus titers, and the latter as evidenced by increased mean survival time.

Table VI-11. Expt. PtA335. Effect of AVS2880 on Intracerebrally Injected Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 15.0 - 16.9 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once daily x 3, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS2880	25	5/5	0.3	1/10	9.7	5.5
	12.5	5/5	1.2	1/10	11.3	5.7
	6.3	5/5	0.7	0/10	9.1	5.4
	3.1	5/5	0.0	0/10	10.8	5.2
	1.5	5/5	0.6	2/10	9.9	6.6
Saline	-	-	-	2/20	11.4	5.9
Normals	-	5/5	0.4	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: Oxamisole exhibited a significant effect against the i.c. PTV infection in a previous experiment (PtA 183), but this was seen at the 1.5 mg/kg/day dose only. A repeat of that study, shown above, failed to confirm that activity.

Table VI-12. Expt. PtA455. Effect of Single Treatment with AVS2933 on Intracerebrally Injected Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 14.0 - 17.1 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain PTV, i.c. injected.
Drug Diluent: Ca^{++}, Mg^{++} free saline.
Treatment Schedule: Once only, beginning 24 hr post-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (ug/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS2933	4800	5/5	1.4	0/10	7.7	6.6
	2400	5/5	-0.1	0/10	8.5	5.6
	1200	5/5	0.1	2/10	7.6	6.8
	600	5/5	0.4	0/10	8.8	6.8
Saline	-	-	-	1/19	7.9	6.4
Normals	-	5/5	0.2	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: CPG 19835 A Lipid was not effective vs the i.c. Balliet PTV infection in this experiment. This treatment schedule was found effective when used against the Adames PTV infections (Expts. PtA 353, 410), although the material was used i.p. in those previous experiments and s.c. in the present study. We will repeat this experiment using i.p. treatment.

Table VI-13. Expt. PtA175. Effect of AVS3588 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 12.9 - 14.1 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily X 3, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Mean Brain Virus[c]
AVS3588	400	5/5	0.7	2/10	10.1	5.1*
	200	5/5	1.5	2/10	10.9	5.7
	100	5/5	1.9	0/10	10.9	6.5
	50	5/5	2.0	1/10	10.7	5.1*
CMC	-	-	-	2/19	10.2	6.2
Normals	-	4/5	2.8	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice [illegible] on or before day 21.

[c]Virus assayed on day 6 [illegible]s inoculation.

*P<0.05 **P<0.01

Conclusions: This is the metafluoro derivative of ABPP, which was previously found to be active vs Adames PTV by this treatment regimen. Moderate activity was seen in the study as determined by reductions in brain virus titers at two concentrations of the test compound.

Table VI-14. Expt. PtA176. Effect of AVS3589 on Intracerebrally Injected Punta Toro Virus Infections in Mice.

Animals: 12.5 - 14.3 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Once daily X 3, beginning 24 hr pre-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Mean Brain Virus[c]
AVS3589	400	5/5	1.9	1/10	10.7	3.8**
	200	5/5	1.6	1/10	9.8	6.5
	100	5/5	1.9	2/10	10.3	6.8
	50	5/5	2.4	2/10	10.1	6.0
CMC	-	-	-	2/10	10.2	6.2
Normals	-	4/5	2.8	-	-	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Virus assayed on day 6 post-virus inoculation.

*P<0.05 **P<0.01

Conclusions: This is 5-chloro-2,3-difluorophenyl derivative of ABPP, which was previously found to be active vs the Adames PTV by this treatment regimen. Moderate activity was seen in the study as determined by reduction in brain virus titers at the highest concentration of the compound used.

Table VI-15. Expt. PtA456. Effect of Twice Daily Treatment with AVS3706 on Intracerebrally Injected Balliet Strain Punta Toro Virus Infections in Mice.

Animals: 14.7 - 16.5 g (4 wk) C57BL/6 Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: 0.4% CMC.
Treatment Schedule: Twice daily x 5, beginning 24 hr pre-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated		
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)	Brain Virus Titers[c]
AVS3706	800	5/5	-1.2	1/10	9.0	6.7
	400	5/5	0.0	0/8	8.6	6.7
	200	5/5	1.1	0/10	9.1	6.7
	100	5/5	0.9	0/10	9.9	6.6
CMC	-	-	-	0/20	8.9	6.5
Normals	-	5/5	1.4	-	-	1.1

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Log_{10} infectious virus recovered from animals killed on infection day 6. Titers determined by viral cytopathic effect in LLC-MK_2 cells.

*P<0.05 **P<0.01

Conclusions: AVS3706 (tiazofurin triacetate) was ineffective against the i.c. PTV infection when used by this treatment regimen.

VII. EFFECT OF A COMBINATION OF AVS01 AND AVS206 ON *IN VITRO* PUNTA TORO VIRUS INFECTIONS

Introduction

As a further effort to define whether AVS206 (ribamidine, ribavirin-3-carboxamidine) might act antivirally in the same manner as AVS01 (ribavirin), an in vitro combination experiment was run to determine if the two compounds might work synergistically. Huggins et al. (1) have shown that ribavirin acts synergistically with the related compounds selenazofurin and tiazofurin in cell culture studies using several RNA viruses. This section describes our *in vitro* experiment.

Materials and Methods

Compounds: AVS01 and AVS206 were provided by Technassociates, Inc. Each was dissolved in test medium, which was MEM containing 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml for use in this experiment.

Cells: Rhesus monkey kidney (LLC-MK_2) cells were used.

Experiment Design: The methodology for our experiment was essentially identical to that described by Huggins et al. (1), in which combinations of AVS01 and AVS206 in fixed ratios of 10:1, 5:1, 2:1, 1:1, 1:2, 1:5 and 1:10 were evaluated vs PTV. Inhibition of CPE in 96-well microplates was used as criterion for evaluation of activity. Seven concentrations of each drug were used at each ratio, each concentration differing by one-half log from the next. Each compound was also run alone at seven concentrations. ED50's for each drug used alone and each combination of drugs were then determined. The activity was then plotted graphically.

As described by several investigators (2-4), if the line of calculated ED50 values fall below the expected line, the combination is considered synergistic. If the calculated points are above the line the action is indifference; i.e., the drugs do not interact or interfere with each other. Antagonism occurs when the ED50 of either drug is greater in combination than it is alone.

In addition to the graphic technique, we also employed the fractional inhibitory concentration (FIC) of each ratio of combination, using the formula:

$$FIC = \frac{\text{ED50 of Drug A in combination}}{\text{ED50 of Drug A alone}} + \frac{\text{ED50 of Drug B in combination}}{\text{ED50 of Drug B alone}}$$

These calculations were done using the ED50 of each individual compound obtained from the experiment. This FIC has been described by Berenbaum (5) and used by Huggins et al. (1) in their combination studies. Allen et al. (6) has interpreted the FIC values as follows:

FIC <0.5:	Significant synergism
FIC 0.5-0.9:	Suggestive of synergism
FIC ~ 1:	Effects are additive
FIC 1.1-1.9:	Indifference or partial antagonism
FIC >2:	Antagonism

Results and Discussion

The graphic representation of this combination experiment is seen in Figure VII-1. In this figure, the ED50 of AVS01 used alone was 6.5 µg/ml, shown on the y-axis. The ED50 for AVS206 was 20 µg/ml, shown on the x-axis. The line between the two ED50 values indicates where the ED50 values would be expected to fall if the compounds are additive to each other in their antiviral action. The ED50 values for each compound at the designated ratios are also plotted, forming the curve above the line drawn between the ED50's. Since the combination ED50 line is above the expected value line, but the ED50's did not exceed those of AVS01 or AVS206 used alone, we conclude that their combined effect was indifferent or they were interfering with each other.

As seen in Figure VII-1, all the FIC values were either additive or in the indifference/partial antagonism range.

Based on these results, we feel AVS01 and AVS206 probably are acting by similar mechanisms, and may be competing somewhat with each other for the same biochemical sites. This possible competition becomes more pronounced as the ratio of AVS206 to AVS01 increases.

Conclusions

AVS01 and AVS206, when used in combination vs Adames PTV in an *in vitro* experiment, appeared to have an indifferent or partial antagonistic effect. These data suggest the compounds are probably acting by similar mechanisms and may be competing with each other.

References

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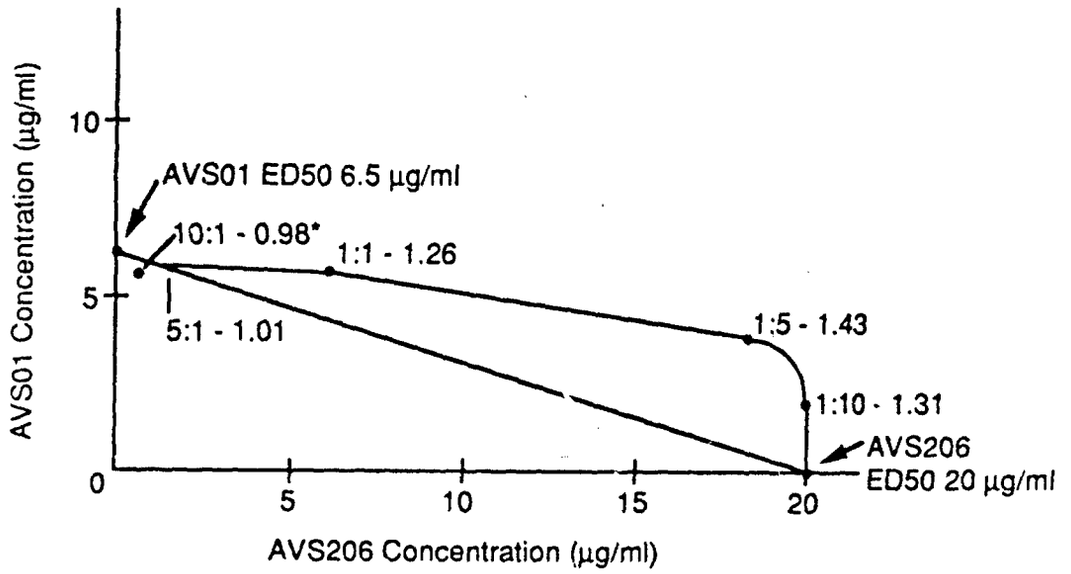
Figure VII-1. PTC653-8. Comparison of Effects of Ribavirin (AVS01) and Ribavirin Carboxamidine (AVS206) Used Alone and in Combination on Punta Toro Virus Infections in LLC-MK_2 Cells.



*Ratio - FIC Index

VIII. EFFECT OF THE COMBINATION OF AVS206 AND AVS2776 ON *IN VIVO* PUNTA TORO VIRUS INFECTIONS

Introduction

As we have previously reported, AVS206, the carboxamidine derivative of ribavirin, has exhibited striking anti-PTV efficacy while being significantly less toxic than ribavirin (AVS01) (1). This compound has therefore been considered as a high priority material for further antiviral evaluations, including being a candidate for possible combination experiments with other anti-PTV materials.

The immunomodulator AVS2776 (bropirimine, ABPP, 2-amino-5-bromo-6-phenyl-4(3H)pyrimidinone) has similarly been considered of great significance because 1) the material was highly active against hepatotropic PTV infections, 2) activity was seen when treatment was oral, and 3) activity was seen when treatment was begun after virus inoculation. This compound was therefore considered a likely candidate for use in combination with AVS206.

An *in vivo* combination chemotherapy experiment was subsequently run using AVS206 and AV2776. The treatment route for each was by oral gavage (p.o.), and the treatment schedule of each was designed to be therapeutic, with AVS206 given twice daily for five days beginning 24 hr post-virus inoculation and AVS2776 given once only 24 hr post-virus inoculation. We have previously shown that each compound was highly active against the in vivo PTV infection using these treatment regimens. Our hypothesis was that the immune modulator at weakly active or inactive dosages would enhance the activity of AVS206 at similarly weak or inactive dosages.

Materials and Methods

Virus: Adames strain PTV as described earlier was used.

Animals: Three week-old C57BL/6 mice (Simonsen) as described previously were used after a 24 hr quarantine. They were maintained on standard mouse chow and tap water *ad libitum*.

Compounds: AVS206 and AVS2776 were provided by Technassociates, Inc. AVS206 was dissolved in sterile water for use in this study; AVS2776, an aqueously insoluble material, was suspended in 0.4% carboxymethylcellulose (CMC).

Experiment Design: A total of five experiments were run in parallel, as follows:

#1 (PtA 287): AVS206 only, p.o., bid x 5 beginning 24 hr post-virus inoculation, at dosages of 75, 37.5, 18.8, 9.4, 4.7, and 2.4 mg/kg/day. These dosages were selected because we previously have shown this material to have weak or no activity below 75 mg/kg/day.

#2 (PtA 291): AVS2776 only, p.o., in a single administration 24 hr post-PTV inoculation, at dosages of 100, 50, and 25 mg/kg. Our earlier work showed AVS2776 to lose its efficacy at doses lower than 100 mg/kg using this p.o. treatment regimen.

#3 (PtA 288): AVS206 at dosages used in #1, + AVS2776 at 100 mg/kg used with each AVS206 dose.

#4 (PtA 289): AVS206 at dosages used in #1, + AVS2776 at 50 mg/kg used with each AVS206 dose.

#5 (PtA 290): AVS206 at dosages used in #1, + AVS2776 at 25 mg/kg used with each AVS206 dose.

An expanded-parameter anti-PTV experiment was run in each experiment, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus titer and serum virus titer, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated infected controls, 20 mice as normal controls and five animals in each treatment group as toxicity controls. One-half of each treatment group, virus controls and normal controls were killed three days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer. Serum was assayed for SGOT, SGPT and PTV virus titers. The remainder of all groups was held 21 days post-virus inoculation with death noted daily.

All methodologies for detecting virus, SGOT, SGPT, etc., and statistical analysis were as done for our standard *in vivo* anti-PTV experiments.

Results and Discussion

The overall results are summarized in Tables VIII-1 to VIII-5. As expected, AVS206 used alone was highly active at the 75 mg/kg/day dose; the lower doses exerted mild effects primarily seen as decreased liver scores and moderately decreased SGOT and SGPT (Table VIII-1). AVS2776 used alone (Table VIII-2) was markedly inhibitory to PTV at the 100 mg/kg dose, although virus titers were not significantly reduced. The 50 mg/kg dose of this immune modulator reduced liver score and transaminase values, but had no effect on survivors or virus titers. The 25 mg/kg dose was not effective using any parameter. Since the 100 mg/kg dose of AVS2776 was so effective, it would be expected that animals treated with this dose in combination with any dose of AVS206 would respond favorably against the infection, and, indeed, this was the case (Table VIII-3). A notable additional observation, however, was the significant reduction in liver and serum virus titers in the animals receiving this high dose of AVS2776 and literally any dose of AVS206, indicating this combination was more effective than either drug used alone. Notable also was the apparent complete toleration by the mice of all doses of the drugs.

The 50 and 25 mg/kg doses of AVS2776 were similarly more effective when used in combination with AVS206 than either drug used alone (Tables VIII-4 and VIII-5). This synergistic effect was seen as increased survivors (Figure VIII-1), decreased virus titers (Figures VIII-2 and VIII-3) and reduction in SGOT and SGPT (Figure VIII-44).

These data indicate that this combination of an antiviral drug (AVS206, ribavirin carboxamidine) and an immunomodulating agent (AVS2776, ABPP) had definite enhanced anti-PTV effect when compared to either substance used alone. When plotted graphically using minimum inhibitory concentration of each compound and statistically significant survivor increase as parameter, this effect appears to be synergistic (Figure VIII-5).

Conclusions

Use of the combination treatments of AVS206 (p.o., bid x 5 beginning 24 hr post-virus inoculation) and AVS2776 (p.o., single treatment 24 hr post-virus inoculation) were more effective than either material used alone for treating in vivo infections with Adames PTV. This combined antiviral effect appeared to be synergistic.

References

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Table VIII-1. Expt. PtA287. Effect of AVS206 Therapy on Punta Toro Virus Infections in Mice (Part I of V in a Combination Experiment with AVS2776).

Animals: 10.9-13.5 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Twice daily x 5, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206	75	5/5	2.9	7/10**	10.7**	1.0**	2/10(2072**)	2/10(1915**)	4.4**	5.1**
	37.5	5/5	3.2	0/10	5.1**	1.1**	1/7(1876**)	1/7(1721**)	4.9**	6.0
	18.8	5/5	3.3	0/10	4.3	1.3**	0/9(4229**)	0/9(4031**)	5.7	6.2
	9.4	5/5	4.0	0/10	4.3	1.8**	0/8(2795**)	0/8(2686**)	5.9	6.5
	4.7	5/5	3.3	0/10	4.0	1.9**	2/9(3192**)	2/9(2694**)	6.1	6.4
	2.4	5/5	3.0	0/10	4.0	2.2*	1/9(6301)	1/9(3498**)	5.5	6.1
H_2O	-	-	-	0/20	4.0	3.0	0/16(10,188)	0/16(8023)	5.6	6.0
Normals	-	5/5	3.2	-	-	0.0	4/5(183)	5/5(34)	0.6	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This is the first experiment in a series to determine the effect of combination therapy with AVS206 (ribavirin carboxamidine) and AVS2776 (ABPP) on PTV infections. In this study, moderate activity was seen down to the lowest dose of AVS206 used, using inhibition of mean liver score as criterion. Mean SGOT and SGPT values were also reduced at these low dosages.

*P<0.05 **P<0.01

Table VIII-2. Expt. PtA291. Effect of AVS2776 Therapy on Punta Toro Virus Infections in Mice (Part II of V in a Combination Experiment).

Animals: 10.9-13.5 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: CMC.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS2776	100	5/5	nr	10/10**	>21.0**	0.2**	10/10**(111**)	9/10**(47**)	4.0	5.1
	50	5/5	nr	0/10	4.1	0.2**	9/10**(119**)	7/10**(111**)	4.1	4.6
	25	5/5	nr	0/10	4.1	1.6	0/9(5430)	0/9(5696)	5.5	6.1
CMC	-	-	-	2/20	4.7	1.8	4/17(4027)	4/17(2945)	4.8	5.3
Normals	-	5/5	3.2	-	-	0.0	4/5(183)	5/5(34)	0.6	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: This is the second experiment in a series to determine the effect of combination therapy with AVS206 and AVS2776 on PTV infections. In this experiment, AVS2776 was used alone, with efficacy seen at 100 and 50 mg/kg.

*P<0.05 **P<0.01

Table VIII-3. Expt. PtA288. Effect of AVS206 and AVS2776 Therapy on Punta Toro Virus Infections in Mice (Part III of V in a Combination Experiment).

Animals: 10.9-13.5 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: 206: H_2O. 2776: CMC.

Treatment Schedule: 206: twice daily x 5, beginning 24 hr post-virus inoculation.
2776: once only, 24 hr post-virus inoculation.

Treatment Route: 206, 2776: p.o.

Expenment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206 +	75 + 100	5/5	3.1	10/10**	>21.0**	0.4**	4/10**(416**)	4/10**(384**)	4.5**	4.8**
AVS2776	37.5 + 100	5/5	2.7	10/10**	>21.0**	0.4**	4/10**(2967*)	2/10*(2983*)	4.8	5.1**
	18.8 + 100	5/5	2.8	4/10**	7.7**	0.7*	5/10**(761**)	4/10**(851**)	3.9**	4.7**
	9.4 + 100	5/5	3.0	8/10**	6.0	0.4**	8/10**(189**)	8/10**(150**)	3.2**	4.7**
	4.7 + 100	5/5	?	5/10**	5.2	0.3**	7/9**(199**)	4/9**(158**)	4.8	5.5**
	2.4 + 100	5/5	3.1	9/10**	4.0	0.8	3/10*(555**)	3/10*(537**)	4.7*	5.9**
H_2O + CMC	-	-	-	0/20	3.9	1.5	1/17(6584)	0/17(7117)	5.8	6.5
Normals	-	5/5	3.2	-	-	0.0	4/5(183)	5/5(34)	0.6	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This is the third experiment in a series to determine the effect of combination therapy with AVS206 and AVS2776 on PTV infections. In this experiment, both drugs were used together, but because AVS2776 at the 100 mg/kg dosage used was relatively active used alone (see part II), little synergism is seen. It is significant, however, that virus titers in spleen and plasma were significantly reduced at dosages of each drug which were not effective against this parameter when used alone.

*P<0.05 **P<0.01

Table VIII-4. Expt. PtA289. Effect of AVS206 and AVS2776 Therapy on Punta Toro Virus Infections in Mice (Part IV of V in a Combination Experiment).

Animals: 10.9-13.5 g (3 wk) C57BL/6 Mice.

Virus: Adames strain Punta Toro virus, s.c. injected.

Drug Diluent: 206: H_2O. 2776: CMC.

Treatment Schedule: 206: twice daily x 5, beginning 24 hr post-virus inoculation. 2776: once only, 24 hr post-virus inoculation.

Treatment Route: 206, 2776: p.o.

Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206 +	75 + 50	5/5	2.8	9/10**	7.0	0.2**	5/9**(654**)	3/9*(643**)	3.4**	4.8**
AVS2776	37.5 + 50	5/5	3.2	3/10**	6.0**	1.4	1/10(4206)	0/10(5626)	4.6**	6.2
	18.8 + 50	5/5	3.2	5/10**	5.2	1.0	1/10(3991)	0/10(4698)	5.5	6.1
	9.4 + 50	5/5	3.0	0/10	4.2*	2.2	0/10(5908)	0/10(5721)	5.0	6.2
	4.7 + 50	5/5	2.3	1/10*	5.2**	1.1	0/10(4423)	0/10(4385)	5.4	6.2
	2.4 + 50	5/5	2.3	4/10**	4.7*	2.7	0/10(8605)	0/10(9150)	6.1	6.3
H_2O + CMC	-	-	-	0/20	3.9	1.5	1/17(6584)	0/17(7117)	5.8	6.5
Normals	-	5/5	3.2	-	-	0.0	4/5(183)	5/5(34)	0.6	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

^AVS206 is lot #12-17-87.

Conclusions: This is the fourth experiment in a series to determine the effect of combination therapy with AVS206 and AVS2776 on PTV infections. In this experiment, both drugs were used together, with definite synergy seen as expressed by increased survivors, increased mean survival times, and reduced virus titers.

*P<0.05 **P<0.01

Table VIII-5. Expt. PtA290. Effect of AVS206 and AVS2776 Therapy on Punta Toro Virus Infections in Mice (Part V of V in a Combination Experiment).

Animals: 10.9-13.5 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 206: H_2O. 2776: CMC.
Treatment Schedule: 206: twice daily x 5, beginning 24 hr post-virus inoculation. 2776: once only, 24 hr post-virus inoculation.
Treatment Route: 206, 2776: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206 +	75 + 25	5/5	3.0	5/10**	5.8	0.9	6/10**(200**)	6/10**(146**)	3.1**	3.5**
AVS2776	37.5 + 25	5/5	3.2	2/10*	5.8	1.9	0/8(4623)	0/8(4546)	5.1	5.8
	18.8 + 25	5/5	2.9	0/10	4.4	1.8	0/9(4776)	0/9(5281)	5.3	6.2
	9.4 + 25	5/5	2.4	0/10	4.6	1.5	1/8(3213)	1/8(3310)	4.8	6.1
	4.7 + 25	5/5	3.8	1/10	4.7	2.4	1/10(5598)	0/10(6875)	4.2**	6.4
	2.4 + 25	5/5	3.1	0/10	4.1	2.2	0/9(6504)	0/9(7667)	4.9	6.3
H_2O + CMC	-	-	-	0/20	3.9	1.5	1/17(6584)	0/17(7117)	5.8	6.5
Normals	-	5/5	3.2	-	-	0.0	4/5(183)	5/5(34)	0.6	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 3 (animals dying prior to day 3 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

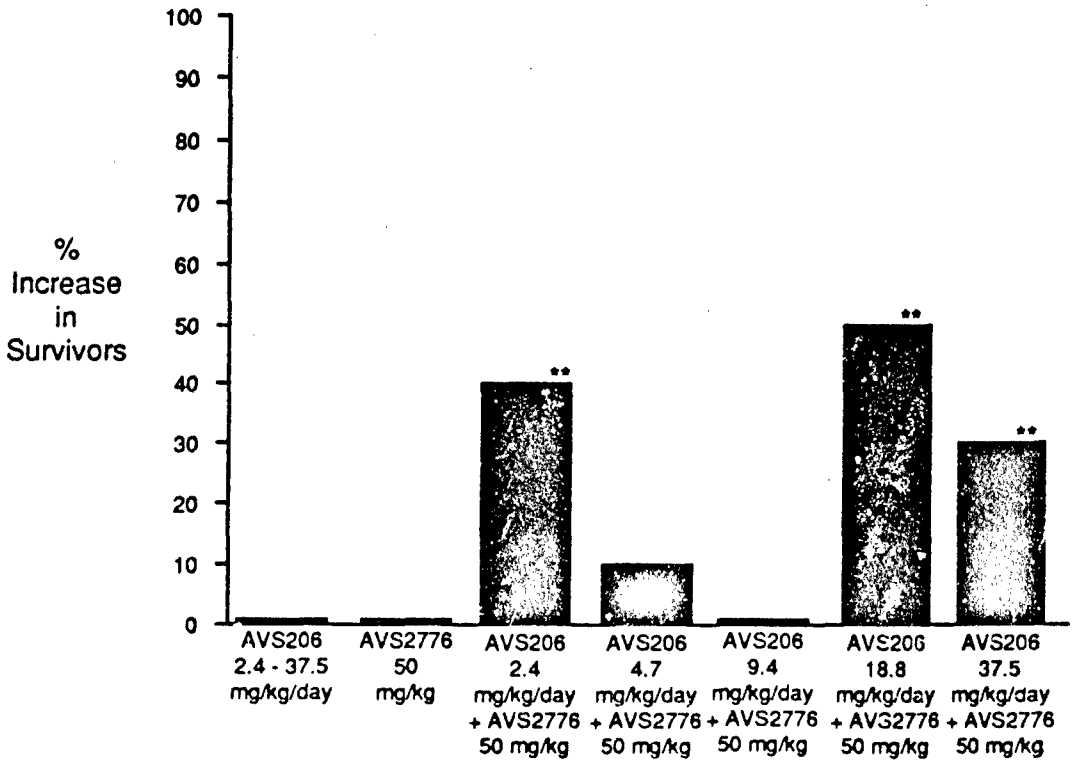
[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This is the fifth experiment in a series to determine the effect of combination therapy with AVS206 and AVS2776 on PTV infections. In this experiment, both drugs were used together, with synergy seen as increased numbers of SGOT and SGPT negatives and in reduced serum and liver virus titers.

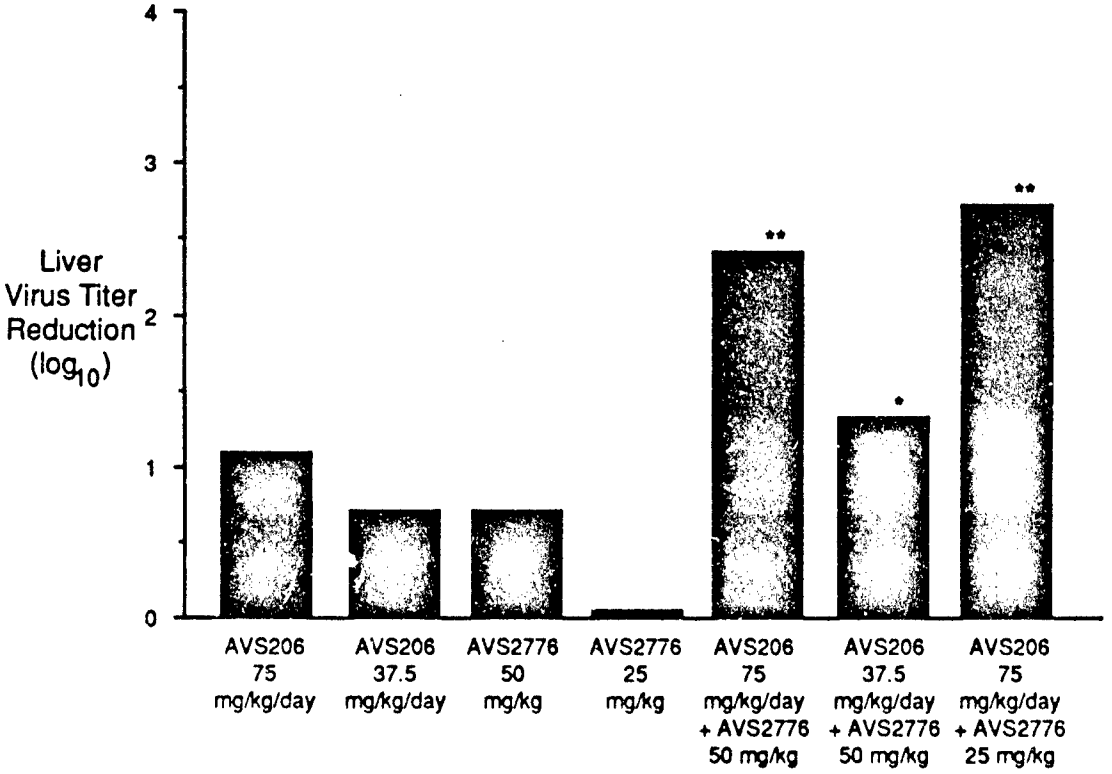
*P<0.05 **P<0.01

Figure VIII-1. Effect of AVS206 (2.4 - 37.5 mg/kg/day) + AVS2776 (50 mg/kg) on Survivor Increases in Infected, Treated Mice.



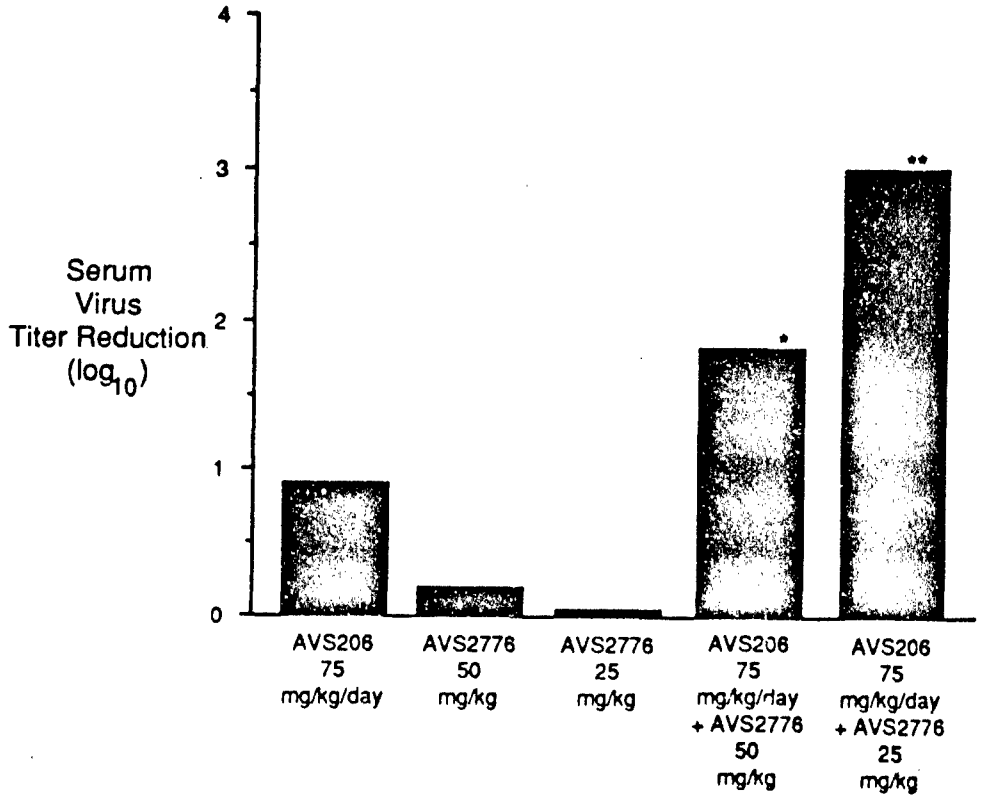
**P <0.01

Figure VIII-2. Effect of AVS206 (37.5 - 75 mg/kg/day) + AVS2776 (50 or 25 mg/kg) on PTV Titer Reduction in Livers of Infected, Treated Mice.



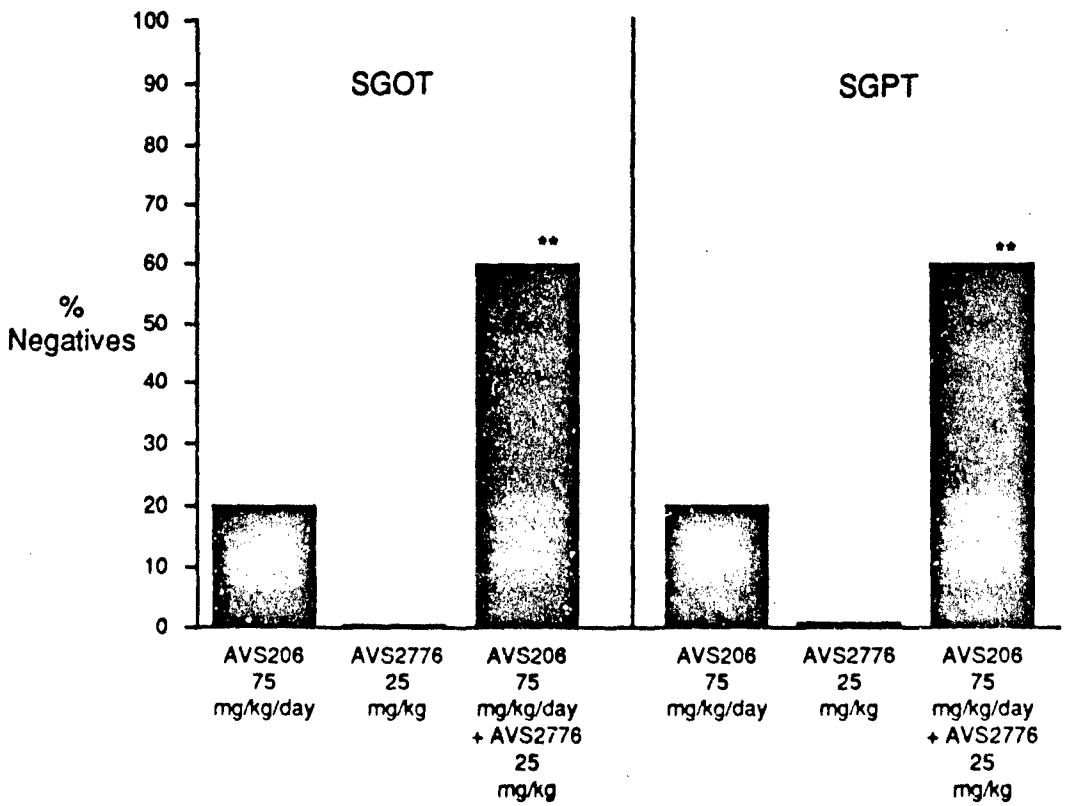
*P < 0.05 **P < 0.01

Figure VIII-3. Effect of AVS206 (75 mg/kg/day) + AVS2776 (50 or 25 mg/kg) on PTV Titer Reduction in Sera of Infected, Treated Mice.



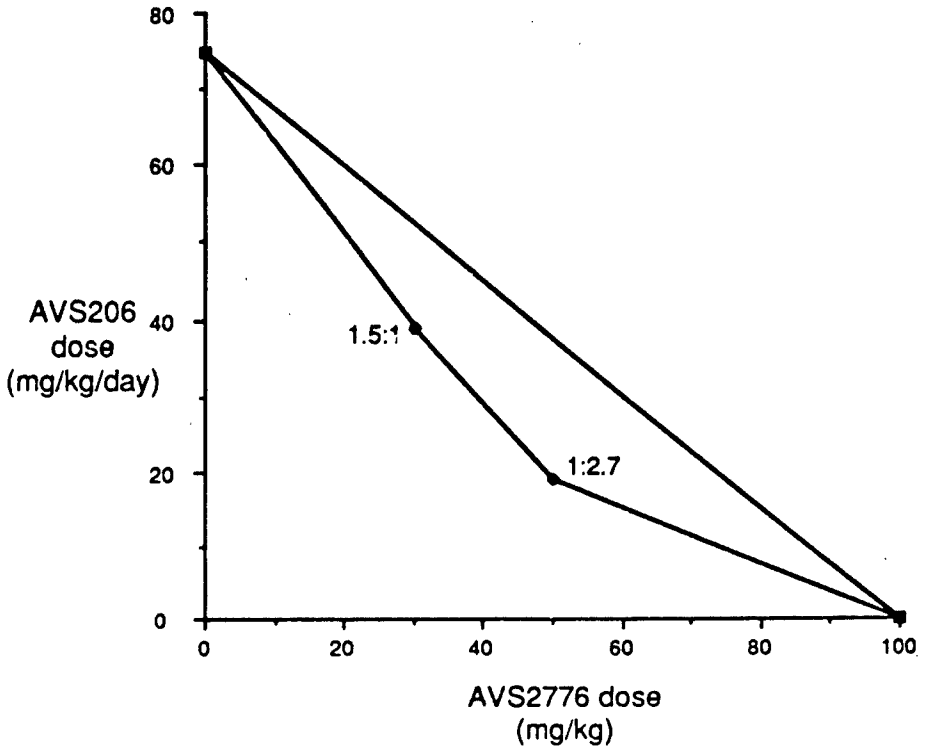
*P <0.05 **P < 0.01

Figure VIII-4. Effect of AVS206 (75 mg/kg/day) + AVS2776 (25 mg/kg) on SGOT and SGPT Reduction in Infected, Treated Mice



**P < 0.01

Figure VIII-5. PtA 287-291. Comparison of Effects of AVS206 and AVS2776 Used Alone and in Combination on PTV Infections in Mice (Survivor Increase Parameter)



IX. EFFECT OF THE COMBINATION OF AVS01 AND AVS1754 ON *IN VIVO* PUNTA TORO VIRUS INFECTIONS

Introduction

As reported previously, AVS01 (ribavirin) has exhibited marked anti-PTV efficacy both *in vitro* and *in vivo* (1). The immunomodulator AVS1754 (MVE-2), a low molecular weight pyran, has also exhibited significant *in vivo* anti-PTV activity, particularly when the material is administered i.p. in a single dose. This section describes an experiment using these two compounds in combination against the in vivo PTV infection.

Materials and Methods

Compounds: AVS01 and AVS1754 were provided by Technassociates, Inc. AVS01 was dissolved in sterile water for oral gavage therapy. AVS1754 was dissolved in sterile physiological saline for i.p. treatment.

Virus: Adames strain PTV as described earlier was used.

Animals: Three week-old C57BL/6 mice (Simonsen) as described previously were used after a 2 hr quarantine. They were maintained on standard mouse chow and tap water *ad libitum*.

Experiment Design: AVS01 was administered p.o. twice daily for 5 days beginning 24 hr post-virus inoculation. AVS1754 was given in a single i.p. injection 24 hr post-virus inoculation. Both treatment regimens with these compounds were previously shown by us to be highly effective against PTV infections in mice. Our hypothesis was that the immunomodulator at weakly active or inactive doses would enhance the activity of AVS01 at similarly weak or inactive dosages. A total of five experiments were run in parallel, as follows:

#1 (PtA427): AVS01 only, at dosages of 200, 32, 10, 3.2, and 1 mg/kg/day. These dosages were selected because we previously have shown this material to have weak or no activity below 32 mg/kg/day.

#2 (PtA431): AVS1754 only, at dosages of 5, 0.5, and 0.05 mg/kg. Our earlier work showed this material to be active only at 5 mg/kg.

#3 (PtA428): AVS01 at doses used in #1 + AVS1754 at 5 mg/kg used with each AVS01 dose.

#4 (PtA429): AVS01 at doses used in #1 + AVS1754 at 0.5 mg/kg used with each AVS01 dose.

#5 (PtA430): AVS01 at doses used in #1 + AVS1754 at 0.05 mg/kg used with each AVS01 dose.

An expanded parameter anti-PTV experiment was run in each experiment, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus titer and serum virus titer, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated infected controls, 20 mice as normal controls and 5 animals in each treatment group as toxicity controls. One-half of each treatment group, virus controls and normal controls were killed 4 days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer. Serum was assayed for SGOT, SGPT and PTV titers. The remainder of the groups were held 21 days post-virus inoculation with deaths noted daily. In this experiment, the total white blood cell (WBC) count was also determined in each animal at the time of sacrifice.

Results and Discussion

The overall results are summarized in Tables IX-1 to 5. AVS01 was highly active at the 200 and 32 mg/kg/day doses, with efficacy seen with all parameters. The 200 mg/kg/day dose was surprisingly well tolerated in this study. Doses below 32 mg/kg/day of AVS01 were not considered efficacious in this study. AVS1754 was moderately effective, reducing mean SGOT and SGPT, liver virus and serum virus titers and elevating WBC levels at the 5 mg/kg dosage only. The lower doses were not effective against the infection.

Combinations of these two compounds were only slightly more effective against the PTV infection than either compound used alone. Possible synergy or additive effects were seen at 32 mg/kg/day and possibly lower doses of AVS01 and 5 mg/kg of AVS1754. These were seen as

increased survivors (Figure IX-1) decreased recoverable titers of liver and serum virus (Figures IX-2 and 3), and decreased SGOT and SGPT values (Figure IX-4).

Conclusions

Treatment of Adames PTV-infected mice with a combination of AVS01 administered p.o. twice daily for 5 days beginning 24 hr after virus inoculation and the immunomodulator AVS1754 administered i.p. in a single injection 24 hr after virus inoculation was considered slightly to moderately more effective than using either compound alone.

References

1. Sidwell, R.W., J.H. Huffman, B.B. Barnett, and D.Y. Pifat. (1988) In vitro and in vivo phlebovirus inhibition by ribavirin. Antimicrob. Ag. Chemother. 32:331-336.

Table IX-1. Expt. PtA427. Effect of Twice Daily p.o. Treatment with AVS01 on Punta Toro Virus Infections in Mice (Part I of a Combination Experiment).

Animals: 12.9-14.2 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Twice daily x 5, beginning 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated							
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})	Total WBC Count[g] ($x10^2/mm^3$)
AVS01	200	5/5	1.4	10/10**	>21.0**	0.3**	4/4**(134**)	4/4**(42**)	1.2**	0.0**	45.5**
	32	5/5	1.5	10/10**	>21.0**	1.4**	1/5(665**)	0/5(1195**)	3.0**	2.3**	17.8*
	10	5/5	1.8	0/10	5.2	3.4	0/3(11,883)	0/3(8167)	5.2	5.9	5.8
	3.2	5/5	2.2	0/10	4.8	3.8	0/4(12,238)	0/4(9025)	5.0	5.9	13.5
	1.0	5/5	1.7	0/10	4.3	3.6	0/5(9820)	0/5(7140)	5.0	6.0	10.8
H_2O	-	-	-	1/20	4.5	3.6	0/9(10,989)	0/9(7578)	4.9	6.0	12.7
Normals	-	5/5	3.1	-	-	0.1	2/2(150)	2/2(47)	0.0	0.0	49.5

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Total white blood cell count. Half of the mice remaining in the B cages on day 4 were used for this and half for serum virus titer, SGOT, and SGPT.

Conclusions: AVS01 (ribavirin) was active as expected in this first part of a combination study. The lowest effective dose was 32 mg/kg/day, although at this dose the total WBC count remained low.

*P<0.05 **P<0.01

Table IX-2. Expt. PtA431. Effect of Single i.p. Treatment with AVS1754 on Punta Toro Virus Infections in Mice (Part II of a Combination Experiment).

Animals: 12.0-14.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile saline.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated							
Compound	Dosage (mg/kg)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})	Total WBC Count[g] ($x10^2/mm^3$)
AVS1754	5	5/5	0.4	3/10	4.9	1.5**	1/7(2521**)	1/7(2351**)	2.6**	2.4**	20.3**
	0.5	5/5	0.4	1/10	5.4	3.3	0/7(11,450)	0/7(9936)	4.8	5.4	12.9
	0.05	5/5	0.5	1/10	4.8	3.4	0/7(14,407)	0/7(12,500)	5.0	6.2	10.8
Saline	-	-	-	1/20	4.8	3.8	0/13(13,041)	0/13(11,088)	5.3	6.0	9.0
Normals	-	5/5	0.6	-	-	0.1	2/2(150)	2/2(47)	0.0	0.0	49.5

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

[g] Total white blood cell count. Some mice from B cages used for this and the rest for serum virus titer, SGOT, and SGPT.

Conclusions: AVS1754 (MVE-2) was moderately effective in this experiment, which was run as a part of a combination study with AVS01. Only the high dose, 5 mg/kg, was effective.

*P<0.05 **P<0.01

Table IX-3. Expt. PtA428. Effect of Twice Daily p.o. Treatment with AVS01 and Single i.p. Treatment with AVS1754 on Punta Toro Virus Infections in Mice (Part III of a Combination Experiment).

Animals: 12.5-13.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 01: H_2O; 1754: Saline.
Treatment Schedule: AVS01: bid x 5, 24 hr post; AVS1754 once only, 24 hr post.
Treatment Route: AVS01: p.o. AVS1754: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated							
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})	Total WBC Count[g] ($x10^2/mm^3$)
AVS01 +	200 + 5	5/5	1.2	10/10**	>21.0**	0.1**	7/7**(90**)	7/7**(50**)	0.3**	0.0**	20.0
AVS1754	32 + 5	5/5	2.6	10/10**	>21.0**	0.7**	5/7**(150**)	2/7(198**)	0.7**	0.3**	13.3
	10 + 5	5/5	1.9	5/10**	5.8*	2.7	0/5(8473)	0/5(3270)	4.3	5.2**	10.9
	3.2 + 5	5/5	2.6	5/10**	5.6*	3.9	0/7(9148)	0/7(3871)	4.9	5.4**	9.3
	1.0 + 5	5/5	1.8	1/10	4.3	3.2	0/5(6618*)	0/5(3270)	3.8*	5.0**	16.3
H_2O + Saline	-	-	-	1/20	4.5	3.6	0/9(10,989)	0/9(7578)	4.9	6.0	12.7
Normals	-	5/5	3.1	-	-	0.1	2/2(150)	2/2(47)	0.0	0.0	49.5

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Total white blood cell count. Some mice from B cages used for this and the rest for serum virus titer, SGOT, and SGPT.

Conclusions: See the comments in Results and Discussion for this section.

*P<0.05 **P<0.01

Table IX-4. Expt. PtA429. Effect of Twice Daily p.o. Treatment with AVS01 and Single i.p. Treatment with AVS1754 on Punta Toro Virus Infections in Mice (Part IV of a Combination Experiment).

Animals: 12.0-14.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 01: H_2O; 1754: Saline.
Treatment Schedule: AVS01: bid x 5, 24 hr post; AVS1754 once only, 24 hr post.
Treatment Route: AVS01: p.o. AVS1754: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated							
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})	Total WBC Count[g] ($x10^2/mm^3$)
AVS01 +	200 + 0.5	5/5	1.3	10/10**	>21.0**	0.4**	7/7**(70**)	7/7**(21**)	0.3**	0.0**	16.2*
AVS1754	32 + 0.5	5/5	2.2	10/10**	>21.0**	2.3**	3/7(1665**)	2/7(3233**)	1.9**	1.9**	31.2**
	10 + 0.5	5/5	2.4	4/10*	6.8*	3.9	0/7(1822**)	0/7(12,500)	5.4	6.5	12.5
	3.2 + 0.5	5/5	1.9	0/10	4.7	4.0	0/7(4871**)	0/7(9479)	5.0	6.1	15.3
	1.0 + 0.5	5/5	1.1	0/10	4.8	2.4**	1/5(3262**)	1/5(6382)	4.0	4.9**	23.9**
H_2O	-	-	-	1/20	4.5	3.6	0/9(10,989)	0/9(7578)	4.9	6.0	12.7
Normals	-	5/5	3.1	-	-	0.1	2/2(150)	2/2(47)	0.0	0.0	49.5

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Total white blood cell count. Some mice from B cages used for this and the rest for serum virus titer, SGOT, and SGPT.

Conclusions: See the comments in Results and Discussion for this section.

*P<0.05 **P<0.01

Table IX-5. Expt. PtA430. Effect of Twice Daily p.o. Treatment with AVS01 and Single I.p. Treatment with AVS1754 on Punta Toro Virus Infections in Mice (Part V of a Combination Experiment).

Animals: 12.0-14.8 g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: 01: H_2O; 1754: Saline.
Treatment Schedule: AVS01: bid x 5, 24 hr post; AVS1754 once only, 24 hr post.
Treatment Route: AVS01: p.o. AVS1754: i.p.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated							
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})	Total WBC Count[g] ($x10^2/mm^3$)
AVS01 +	200 + 0.05	5/5	1.3	10/10**	>21.0**	0.0**	7/7**(145**)	7/7**(50**)	0.6**	0.3**	14.1
AVS1754	32 + 0.05	5/5	2.3	9/10**	7.0	1.8**	0/6(4503**)	0/6(3467)	3.8*	2.7**	11.4
	10 + 0.05	5/5	2.8	1/10	5.6	3.6	0/6(19,325)	0/6(11,458)	5.2	6.3	6.8
	3.2 + 0.05	5/5	1.8	1/10	5.2	3.2	0/5(18,170)	0/5(10,660)	4.8	5.8	12.5
	1.0 + 0.05	5/5	2.3	0/10	4.7	3.7	0/7(18,114)	0/7(11,714)	5.2	6.4	6.0
H_2O	-	-	-	1/20	4.5	3.6	0/9(10,989)	0/9(7578)	4.9	6.0	12.7
Normals	-	5/5	3.1	-	-	0.1	2/2(150)	2/2(47)	0.0	0.0	49.5

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

[g]Total white blood cell count. Three mice from B cages used for this and the rest for serum virus titer, SGOT, and SGPT.

Conclusions: See the comments in Results and Discussion for this section.

*P<0.05 **P<0.01

Figure IX-1. Effect of Treatment with the Combination of AVS01 (3.2-10 mg/kg/day) and AVS1754 (5 mg/kg) on Survivor Increases in PTV-Infected Mice.

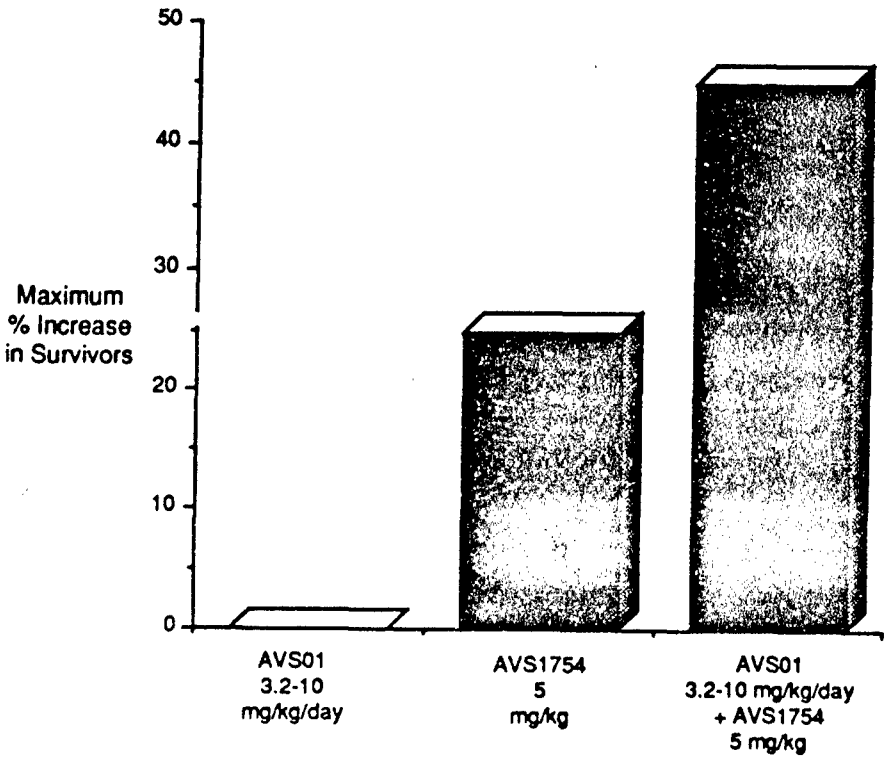


Figure IX-2. Effect of Treatment with the Combination of AVS01 (32 mg/kg/day) and AVS1754 (5 mg/kg) on Reduction in Liver and Serum Virus Titers in PTV-Infected Mice.

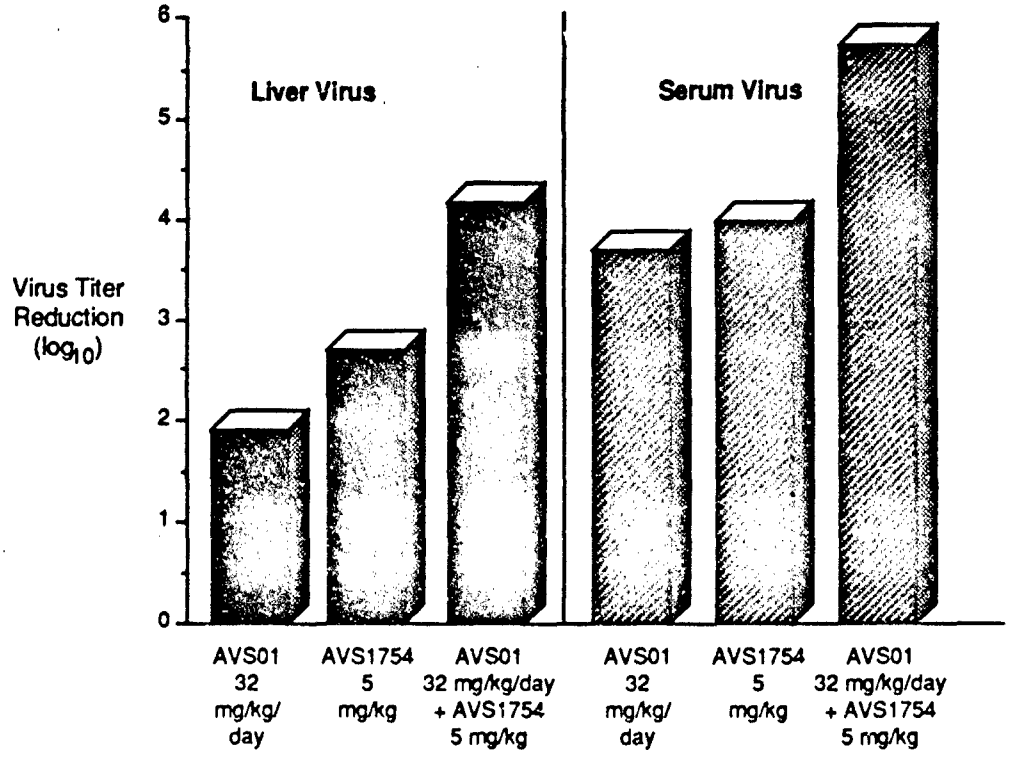


Figure IX-3. Effect of Treatment with the Combination of AVS01 (32 mg/kg/day) and AVS1754 (0.5 mg/kg) on Reduction in Liver and Serum Virus Titers in PTV-Infected Mice.

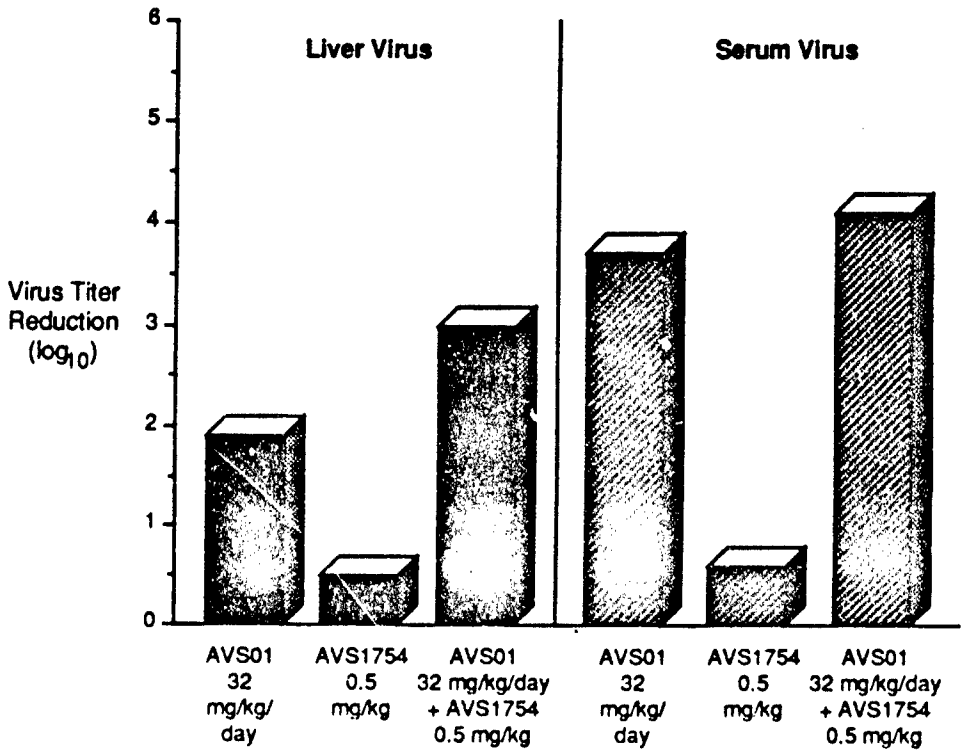
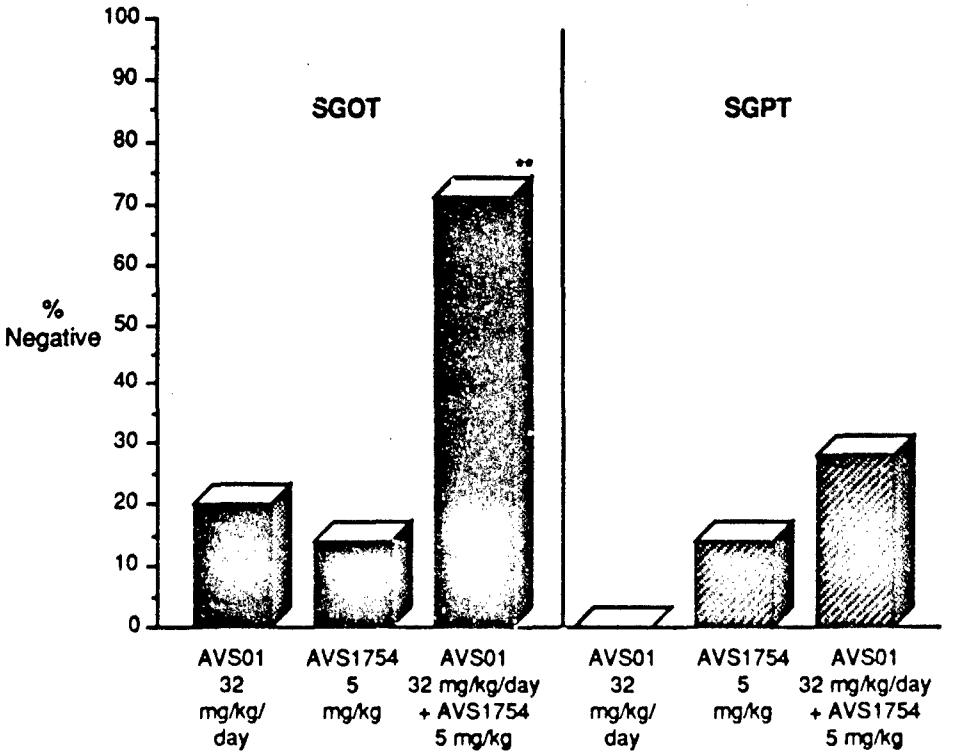


Figure IX-4. Effect of Treatment with the Combination of AVS01 (32 mg/kg/day) and AVS1754 (5 mg/kg) on Increase in Serum Samples Having Negative[a] SGOT and SGPT Values.



**P<0.05

[a]SGOT levels of <200 Sigma-Fraenkel units/ml, SGPT levels of <100 Sigma-Fraenkel units/ml

X. EFFECT OF A COMBINATION OF AVS206 AND AVS1767 ON IN VIVO PUNTA TORO VIRUS INFECTIONS

Introduction

As we have previously reported, AVS206 (ribavirin 3-carboxamidine, ribamidine) has exhibited striking anti-PTV efficacy while being significantly less toxic than ribavirin (AVS01) (1). This compound has therefore been considered a high priority material for further antiviral evaluations, including being a candidate for possible combination experiments with other anti-PTV materials.

The immunomodulator AVS1767 (AM-3) has similarly been considered of great significance as a means of treatment of PTV infections. The compound, a fungal product developed by Spanish investigators, reportedly enhances interferon induction (2), natural killer cell activity (2, 3), lymphocyte proliferation (3), interleukin-2 production (3), T-cell mitogen response (3, 4) and to restore antibody responses and delayed hypersensitivity reactions (5). This compound was therefore considered a likely candidate for use in combination with AVS206.

This section describes the results of using these two materials in combination vs PTV infections. With each compound, a therapeutic treatment regimen was used which we have previously found to be highly effective against this virus infection. Dosages of each compound were selected to bracket those previously found effective and those marginally or ineffective in order to more fully demonstrate if the combination would result in a greater effect than using either material at that dosage alone.

Materials and Methods

Compounds: AVS206 and AVS1767 were provided by Technassociates, Inc. AVS206 was dissolved in sterile water for p.o. therapy. AVS1767 was dissolved in sterile physiological saline for s.c. treatment.

Virus: The Adames strain of PTV as previously described was used.

Animals: Female 3 week-old C57BL/6 mice (Simonsen) as described previously were used after a 24 hr quarantine. They were maintained on standard mouse chow and tap water *ad libitum.*

Experiment Design: AVS206 was administered p.o., twice daily for 5 days beginning 18 hr post-virus inoculation. AVS1767 was given in a single s.c. injection 48 hr post-virus inoculation. Both treatment regimens with these compounds were previously shown by us to be highly effective against PTV infections in mice. Our hypothesis was that the immune modulator at weakly active or inactive dosages would enhance the activity of AVS206 at similarly weak or inactive dosages. A total of five experiments were run in parallel, as follows:

#1 (PtA 382): AVS206 only, at dosages of 75, 37.5, 18.8, 9.4, 4.7, and 2.4 mg/kg/day. These dosages were selected because we previously have shown this material to have weak or no activity below 37.5 mg/kg/day.

#2 (PtA 386): AVS1767 only, at dosages of 50, 16 and 5 mg/kg. Our earlier work showed AVS1767 to lose its efficacy at doses lower than 50 mg/kg.

#3 (PtA 383): AVS206 at doses used in #1, + AVS1767 at 50 mg/kg used with each AVS206 dose.

#4 (PtA 384): AVS206 at doses used in #1, + AVS1767 at 16 mg/kg used with each AVS206 dose.

#5 (PtA 385): AVS206 at doses used in #1, + AVS1767 at 5 mg/kg used with each AVS206 dose.

An expanded parameter anti-PTV experiment was run in each experiment, the disease parameters being survivors, mean survival time, liver score, SGOT, SGPT, liver virus titer and serum virus titer, with 20 infected mice used in each treatment group, 40 mice used as placebo-treated infected controls, 20 mice as normal controls and 5 animals in each treatment group as toxicity controls. One-half of each treatment group, virus controls and normal controls were killed 4 days after virus inoculation, bled, and their livers removed. Livers were scored from 0 to 4, homogenates prepared from each, and the homogenates tested for virus titer. Serum was assayed for SGOT, SGPT and PTV titers. The remainder of the groups were held 21 days post-virus inoculation with deaths noted daily.

Results and Discussion

The overall results are summarized in Tables X-1 to 5. As expected, AVS206 used alone (Table X-1) was highly active at the 75 mg/kg/day dose; the 37.5 mg/kg/day dose was moderately effective only in preventing death in 50% of the mice and prolonging mean survival time. The 18.8 mg/kg/day dose was less effective yet, with only a 20% survivor increase and significant mean survival time increase. AVS1767 was less effective than has been seen previously when used alone (Table X-2). At the maximum dose used, only a significant decrease in mean liver virus titer was seen. Combinations of the two compounds had definite synergistic effects (Tables X-3-5). These were seen as reductions in liver score (Figure X-1), reductions in SGOT and SGPT (Figure X-2), inhibition of serum PTV titers (Figures X-3-5) and inhibition of liver PTV titers (Figures X-6-7).

These data indicate this combination of an antiviral drug (AVS206) and an immunomodulating agent (AVS1767) had a definite enhanced anti-PTV effect when compared to either substance used alone. The combinations used were apparently well tolerated, since the weight gains in the various toxicity controls receiving AVS206 or AVS206 + AVS1767 were similar.

Conclusions

Treatment with AVS206 administered p.o. twice daily for 5 days beginning 18 hr after virus inoculation combined with the immunomodulator AVS1767 injected s.c. a single time 48 hr after virus inoculation had a definite enhanced effect on *in vivo* Adames PTV infections. This was seen as reductions in liver score, SGOT, SGPT, serum virus titers and liver virus titers.

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Table X-1. Expt. PtA382. Effect of Twice Daily p.o. Treatment with AVS206 on Punta Toro Virus Infections in Mice (Part 1 of a Combination Study with AVS1767).

Animals: 12.5-14.3g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O.
Treatment Schedule: Twice daily x 5, beginning 18 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206	75	5/5	1.5	10/10**	>21.0**	2.6**	2/10(890**)	1/10(1017**)	3.0**	1.9**
	37.5	5/5	1.8	5/10**	6.4**	3.3	1/10(4995)	0/10(5177)	4.3	4.6
	18.8	5/5	2.2	2/10*	7.3**	3.6	0/9(10378)	0/9(10244)	5.1	5.9
	9.4	5/5	2.8	0/10	4.5	3.8	0/9(13000)	0/9(12500)	5.7	6.4
	4.7	5/5	3.0	0/10	4.3	3.2	1/10(10079)	1/10(9091)	5.1	5.6
	2.4	5/5	1.3	0/10	4.6	3.5	0/10(7662)	0/10(8155)	4.9	5.6
H_2O	-	-	-	0/20	4.6	3.2	3/19(6249)	2/19(7594)	4.7	5.5
Normals	-	5/5	2.6	-	-	0.3	4/5(109)	5/5(24)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This is the first experiment in a series to determine the effects of combination therapy with AVS206 (ribamidine) and AVS1767 (AM-3). In this experiment, ribamidine used alone was active vs PTV at doses down to 18.8 mg/kg/day.

*P<0.05 **P<0.01

Table X-2. Expt. PtA386. Effect of s.c. Treatment with AVS1767 on Punta Toro Virus Infections in Mice (Part 2 of a Combination Study).

Animals: 12.0-14.0g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Saline.
Treatment Schedule: Once only, 48 hr post-virus inoculation.
Treatment Route: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected, Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS1767	50	5/5	0.6	3/10	5.3	3.4	0/10(5045)	0/10(6195)	4.2**	5.4
	16	5/5	0.7	0/10	4.7	3.8	0/10(8643)	0/10(10281)	5.1	6.1
	5	5/5	0.4	1/10	5.4	3.7	0/9(9550)	0/9(11333)	5.4	6.5
Saline	-	-	-	7/20	5.3	3.5	1/17(6882)	0/17(8026)	5.1	5.7
Normals	-	5/5	0.5	-	-	0.3	4/5(109)	5/5(24)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: This is the second experiment in a series to determine the effects of combination therapy with ribamidine and AM-3 on PTV infections. In this experiment, AM-3 was only weakly effective, although a previous experiment (PtA 263) indicated this treatment regimen was highly effective with this immunomodulator.

*P<0.05 **P<0.01

Table X-3. Expt. PtA383. Effect of a Combination of AVS206 and AVS1767 on Punta Toro Virus Infections in Mice (Part 3 of a Combination Study).

Animals: 12.0-14.0g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O + Saline.
Treatment Schedule: 206: bid x 5, 18 hr post; 1767 once only, 48 hr post.
Treatment Route: 206: p.o.; 1767: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206 +	75 + 50	5/5	1.0	10/10**	>21.0**	0.4**	7/9**(165**)	5/9**(143**)	0.3**	2.3**
AVS1767	37.5 + 50	5/5	2.0	7/10**	6.0	1.1**	2/10(1323**)	0/10(3439**)	3.7*	4.6**
	18.8 + 50	5/5	1.8	3/10*	5.7	3.1	1/9(3438**)	2/9(3849**)	3.4**	4.4**
	9.4 + 50	5/5	2 3	1/10	4.7	2.8	0/10(6482)	0/10(8442)	4.5	5.8
	4.7 + 50	5/5	1.8	0/10	5.2	2.3**	1/9(4184**)	0/9(5845*)	3.9*	5.3*
	2.4 + 50	5/5	2.0	0/10	5.3	3.9	0/10(8939)	0/10(10295)	5.3	6.2
H_2O + Saline	-	-	-	0/20	4.8	3.7	0/19(8277)	0/19(9561)	4.9	6.2
Normals	-	5/5	2.6	-	-	0.3	4/5(109)	5/5(24)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This is the third experiment in a series to determine the effects of combination therapy with ribamidine and AM-3. The combined therapy resulted in more liver score inhibition, greater reductions in SGOT and SGPT, and less recoverable liver and serum virus titers than with either compound used alone.

*P<0.05 **P<0.01

Table X-4. Expt. PtA384. Effect of a Combination of AVS206 and AVS1767 on Punta Toro Virus Infections in Mice (Part 4 of a Combination Study).

Animals: 12.0-14.0g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O + Saline.
Treatment Schedule: 206: bid x 5, 18 hr post; 1767 once only, 48 hr post.
Treatment Route: 206: p.o.; 1767: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206 +	75 + 16	5/5	2.8	10/10**	>21.0**	0.3**	6/10**(497**)	6/10**(246**)	1.1**	2.7**
AVS1767	37.5 + 16	5/5	2.8	9/10**	5.0	0.7**	0/9(2114**)	0/9(600**)	1.7**	2.0**
	18.8 + 16	5/5	1.2	5/10**	6.0	1.2**	0/10(8272)	0/10(2580**)	4.3	4.6**
	9.4 + 16	5/5	2.4	0/10	5.2	1.5**	0/9(6462)	0/9(5983)	3.9*	4.2**
	4.7 + 16	5/5	1.9	0/10	5.1	2.1**	0/9(9789)	0/9(7778)	4.8	5.8
	2.4 + 16	5/5	1.0	0/10	5.3	3.6	0/10(9104)	0/10(7872)	4.7	5.5
H_2O + Saline	-	-	-	0/20	5.7	3.7	0/19(8277)	0/19(9561)	4.9	6.2
Normals	-	5/5	2.6	-	-	0.3	4/5(109)	5/5(24)	0.0	0.0

[a] Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b] Mean survival time of mice dying on or before day 21.

[c] Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d] Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

[e] Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f] Geometric mean.

Conclusions: This is the fourth experiment in a series to determine the effects of combination therapy with ribamidine and AM-3. The combined therapy resulted in more liver score inhibition, greater reductions in SGOT and SGPT, and less recoverable liver and serum virus titers than with either compound used alone.

*P<0.05 **P<0.01

Table X-5. Expt. PtA385. Effect of a Combination of AVS206 and AVS1767 on Punta Toro Virus Infections in Mice (Part 5 of a Combination Study).

Animals: 12.0-14.0g (3 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: H_2O + Saline.

Treatment Schedule: 206: bid x 5, 18 hr post; 1767 once only, 48 hr post.
Treatment Route: 206: p.o.; 1767: s.c.
Experiment Duration: 21 days.

		Toxicity controls		Infected Treated						
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change[a] (g)	Surv/ Total	MST[b] (days)	Mean Liver Score[c]	SGOT Neg/Total[d] (Mean)	SGPT Neg/Total[e] (Mean)	Mean Liver Virus Titer[f] (log_{10})	Mean Serum Virus Titer[f] (log_{10})
AVS206 +	75 + 5	5/5	2.2	5/10**	5.6	0.7**	5/10**(249**)	0/10(1141**)	1.0**	1.4**
AVS1767	37.5 + 5	5/5	1.7	7/10**	5.0	1.7**	1/10(2041**)	0/10(3342**)	3.0**	3.1**
	18.8 + 5	5/5	1.9	0/10	5.0	2.8	0/8(5719)	0/8(5875)	4.6	5.6
	9.4 + 5	5/5	2.5	0/10	4.9	3.0	0/9(9306)	0/9(7087)	5.0	6.0
	4.7 + 5	5/5	1.1	0/10	4.6	4.0	0/10(10727)	0/10(8215)	5.2	6.3
	2.4 + 5	5/5	1.9	0/10	4.8	3.8	0/9(12800)	0/9(10050)	5.4	6.4
H_2O + Saline	-	-	-	0/20	5.7	3.7	0/19(8277)	0/19(9561)	4.9	6.2
Normals	-	5/5	2.6	-	-	0.3	4/5(109)	5/5(24)	0.0	0.0

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day 4 (animals dying prior to day 4 assigned a liver score of 4).

[d]Serum glutamic oxalic transaminase levels of <200 Sigma-Fraenkel units/ml.

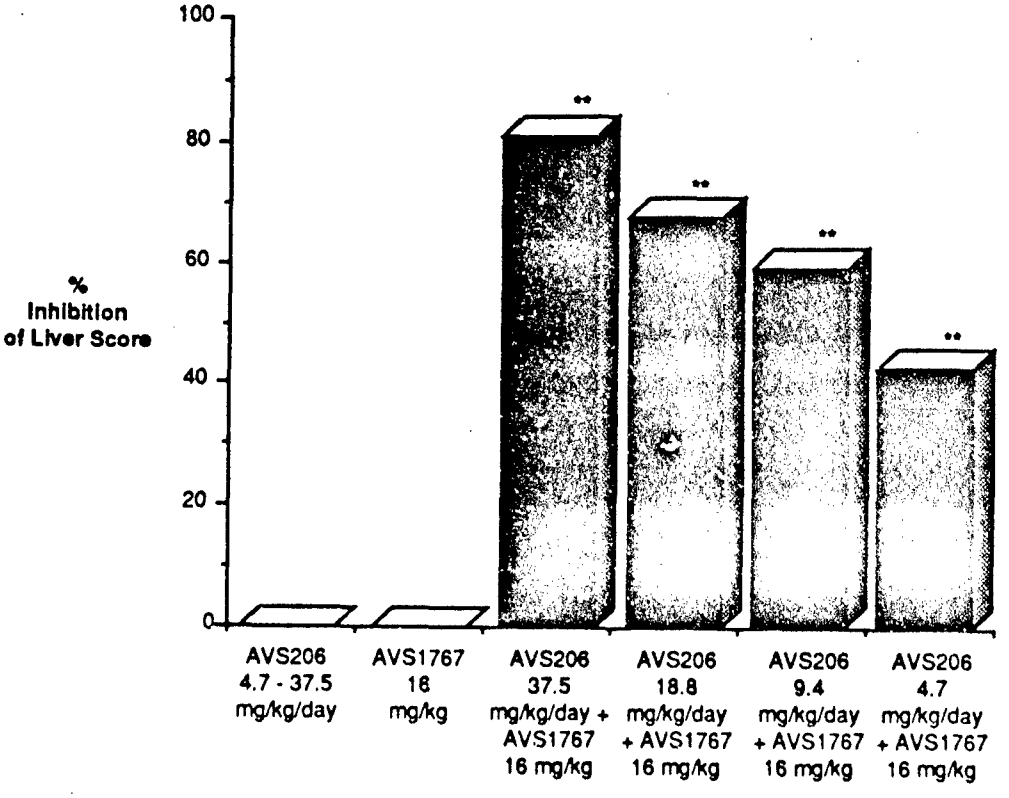
[e]Serum glutamic pyruvic transaminase levels of <100 Sigma-Fraenkel units/ml.

[f]Geometric mean.

Conclusions: This is the fifth experiment in a series to determine the effects of combination therapy with ribamidine and AM-3. The combined therapy resulted in more liver score inhibition, greater reductions in SGOT and SGPT, and less recoverable liver and serum virus titers than with either compound used alone.

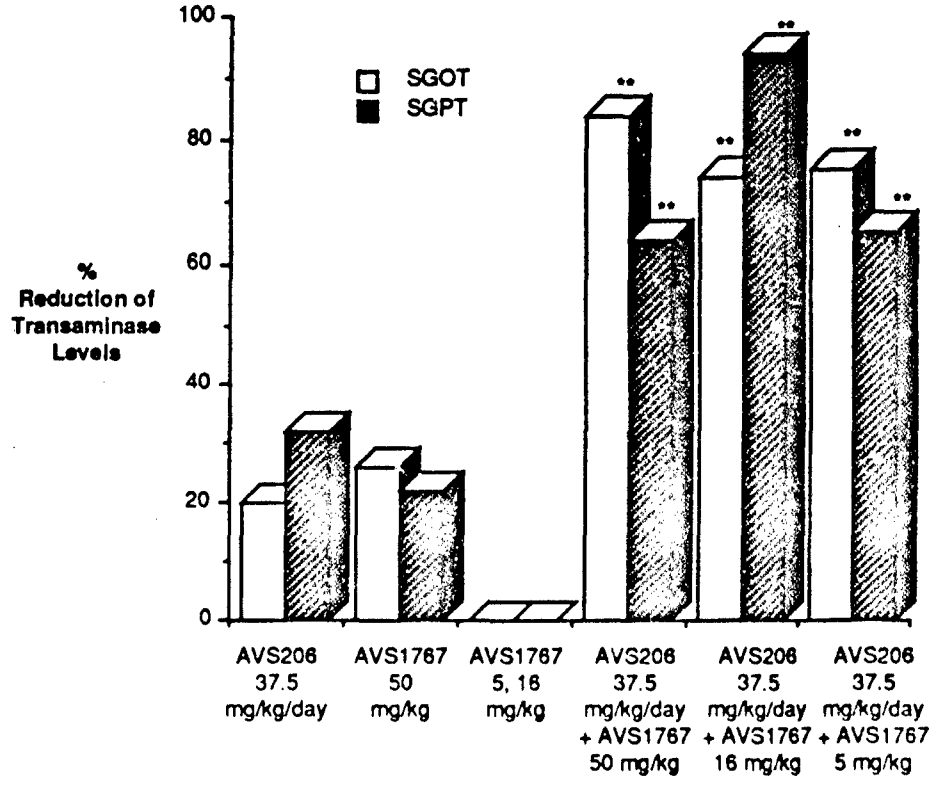
*P<0.05 **P<0.01

Figure X-1. Effects of the Combination of AVS206 (4.7 - 37.5 mg/kg/day) + AVS1767 (16 mg/kg) on Reduction of Liver Score in PTV-Infected Mice.



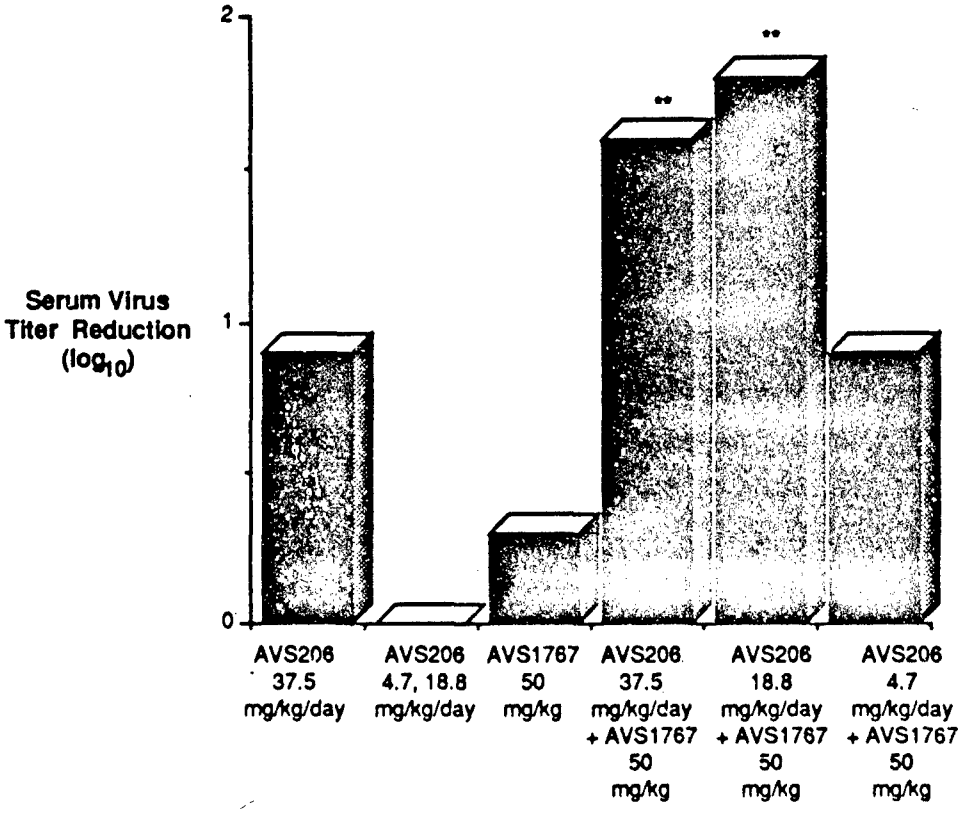
**P<0.01

Figure X-2. Effects of the Combination of AVS206 (37.5 mg/kg/day) + AVS1767 (5, 16, 50 mg/kg) on Reduction of SGOT and SGPT in PTV-Infected Mice



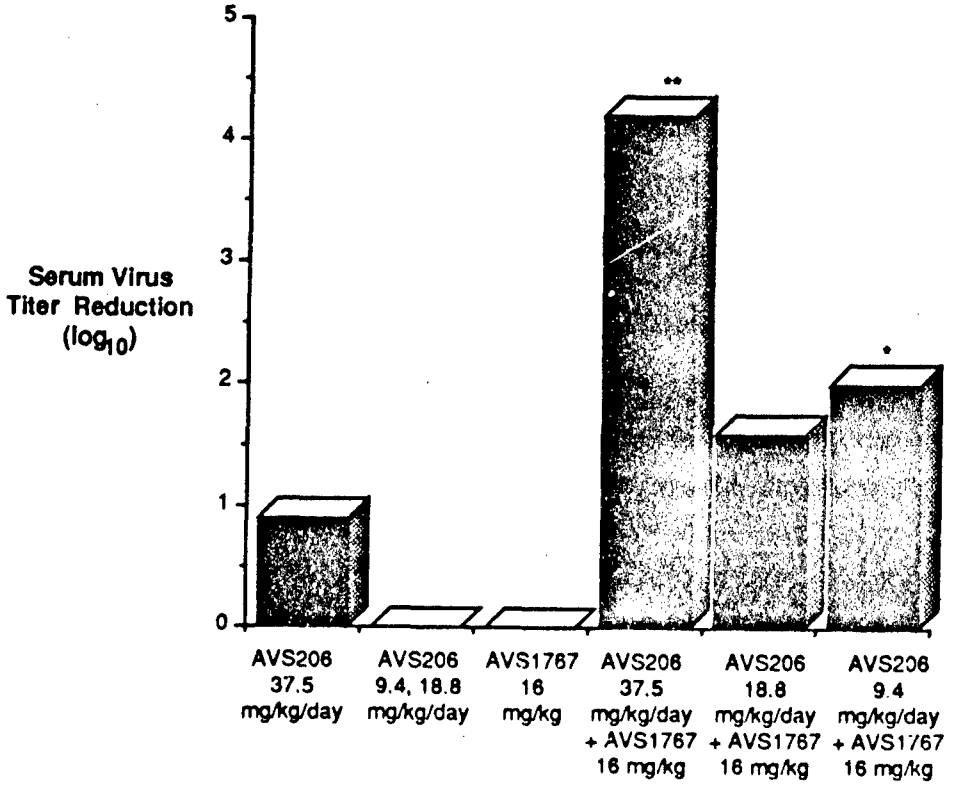
**P<0.01

Figure X-3. Effect of the Combination of AVS206 (4.7, 18.8, 37.5 mg/kg/day) + AVS1767 (50 mg/kg) on Serum Virus Titer Reductions on PTV Infected Mice.



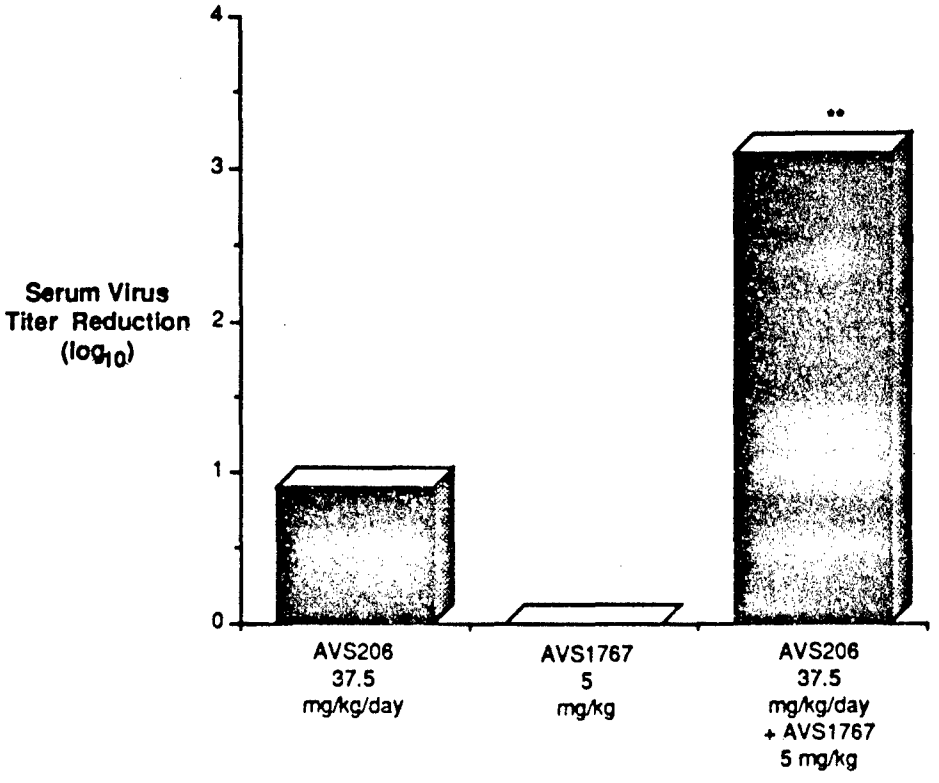
**P<0.01

Figure X-4. Effect of the Combination of AVS206 (9.4 - 37.5 mg/kg/day) + AVS1767 (16 mg/kg) on Serum Virus Titer Reductions in PTV Infected Mice.



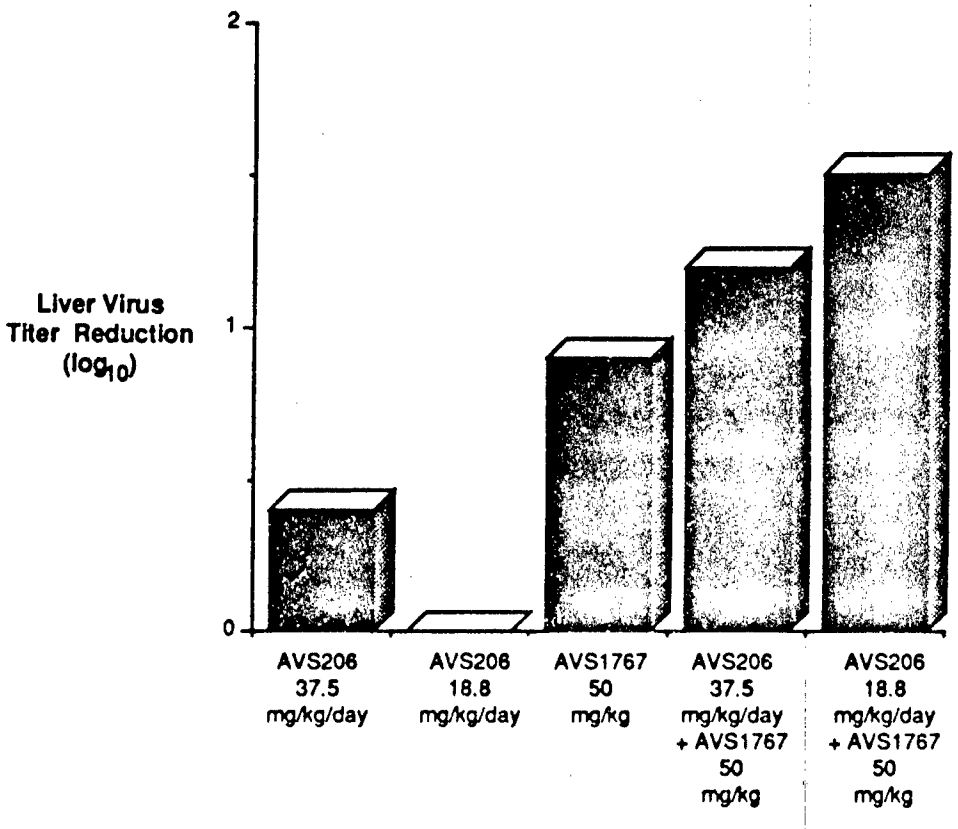
*P<0.05 **P<0.01

Figure X-5. Effect of the Combination of AVS206 (37.5 mg/kg/day) + AVS1767 (5 mg/kg) on Serum Virus Titer Reductions in PTV Infected Mice



**P<0.01

Figure X-6. Effect of the Combination of AVS206 (18.8, 37.5 mg/kg/day) + AVS1767 (50 mg/kg) on Liver Virus Titer Reductions on PTV Infected Mice.



XI. EFFECTS OF TREATMENT WITH AVS206 ON DELAYED INFECTION PARAMETERS IN PUNTA TORO VIRUS-INFECTED MICE

Introduction

During a seminar presented by us to USAMRIID personnel in July, 1988, it was suggested to us that we should also determine if AVS206 (ribamidine, ribavirin carboxamidine) antiviral effects were seen if animals were assayed at later times in the infection. An experiment was therefore run to investigate this.

Materials and Methods

Compound: AVS206 was provided by Technassociates, Inc for this study. The compound was dissolved in sterile water for this study.

Virus: The Adames strain of PTV as previously described was used.

Animals: Female 3 week-old C57BL/6 mice (Simonsen) as described previously were used after a 24 hr quarantine.

Experiment Design: Mice were infected s.c. with a standard inoculum of PTV, then treated p.o. twice daily for 3 days with 1000 mg/kg/day of AVS206 beginning 24 hr post-virus inoculation. Five infected, AVS206-treated and H_2O-treated mice were then killed on infection days 3, 4, 5, 6, and 7. The livers were given scores of 0 to 4 for hepatic icterus and the livers and sera were individually assayed for virus titer by our standard procedure using LLC-MK_2 cells. Mice dying before sacrifice were assumed to have 4+ liver scores and maximal liver and serum virus titers.

All assays for virus were as described for our standard *in vivo* anti-PTV experiments

Results and Discussion

The results of this study are seen in Table XI-1. On day 3, all livers from mice treated with AVS206 appeared normal and no virus was recovered from their livers or sera. The H_2O-treated infected animals had a relatively low liver score but high virus titers in both livers and sera. By day 4, the virus control mice had markedly discolored livers and high titers of virus recovered from livers and sera. The AVS206-treated mice had slight (mean 0.4) liver discoloration and low ($10^{0.5}$) virus in their liver but none in their serum. The liver scores of the AVS206-treated mice declined to 0 through the remainder of the experiment, although moderate (less then $10^{2.0}$) virus levels were seen in the livers and sera from the mice on day 5. No virus was seen in livers or serum samples from these animals on days 6 and 7. All of the virus control mice had died by day 5.

We conclude that treatment with AVS206 was highly effective in reducing the infection in the mice, and, once treatment terminated by the end of day 3, the infection did not return to any significant extent.

Conclusions

AVS206 administered p.o. twice daily for 3 days was highly effective vs Adames PTV infections in mice, and, once treatment was terminated, detectable infectious virus did not return to either livers or sera from surviving mice up to 4 days later.

Table XI-1. Expt. PtA447. Effect of AVS206 on Punta Toro Virus Infections in Mice When Assayed at Varying Days Post-Virus Inoculation.

Animals: 11.6-13.0 g (3-4 wk) C57BL/6 Mice.
Virus: Adames strain Punta Toro virus, s.c. injected.
Drug Diluent: Sterile H_2O.
Treatment Schedule: Twice daily x 3, 24 hr post-virus inoculation.
Treatment Route: p.o.
Experiment Duration: 21 days.

Compound	Experiment Day[a]	Mean Liver Score[b]	Mean Liver Virus Titer (log_{10})	Mean Serum Virus Titer (log_{10})
AVS206[c]	3	0.0**	0.0**	0.0**
H_2O		1.2	5.4	6.2
AVS206	4	0.4**	0.5**	0.0**
H_2O		3.1	4.0	5.0
AVS206	5	0.0**	1.8**	1.1**
H_2O[d]		4.0	5.7	6.5
AVS206	6	0.0**	0.0**	0.7**
H_2O		4.0	5.7	6.5
AVS206	7	0.0**	0.0**	0.0**
H_2O		4.0	5.7	6.5

[a]Days post-virus inoculation on which five animals were sacrificed.

[b]Scores of 0 (normal liver) to 4 (maximal discoloration) assigned to each liver removed on day of sacrifice (animals dying prior to sacrifice assigned a liver score of 4).

[c]AVS206 concentration was 1000 mg/kg/day. Toxicity controls all survived and gained 0.6 g as compared to 1.2 g in normal controls.

[d]All H_2O controls were dead by day 5 of experiment, therefore all animals were given the highest score observed before this time.

**$P<0.01$ as compared with the corresponding H_2O control animals.

Conclusions: Ribamidine had previously been shown to markedly affect PTV infections, with inhibition of serum and liver virus titers and mean liver score seen at 2 or 3 days post-virus inoculation. The present study was run to determine if this therapy maintained a low score and virus titer if assayed at later times. The data indicate slight rises in virus titer by day 5, but this subsided again by days 6 and 7.

XII. INFECTIVITY OF BALLIET STRAIN PTV INOCULATED INTRACEREBRALLY INTO BALB/C AND SWISS WEBSTER MICE.

Introduction

We had been instructed by our COTR to initiate studies into the effect of intravenous treatment of mice infected intracerebrally (i.c.) with the neurotropic PTV. Our previous work had involved the use of C57BL/6 mice, which are black; because of this dark color they are difficult to inoculate intravenously (i.v.), so we therefore decided to use a susceptible strain of white mice to more readily accomplish this method of treatment. This section describes the infectivity of the neurotropic PTV in two strains of white mice.

Materials and Methods

Animals: Four week-old female Balb/c and Swiss Webster mice were obtained from Simonsen Laboratories. The animals were quarantined 48 hr prior to use and were maintained on standard mouse chow and water *ad libitum*.

Virus: The Balliet strain of PTV was used in this study.

Experiment Design: Ten mice of each strain were anesthetized with ether and inoculated i.c. with each of 8 ten-fold dilutions of virus. The animals were held through day 21 or until death and 50% lethal doses (LD50) determined.

Results and Discussion

The results of this titration are seen in Table XII-1. Both strains of mice were readily susceptible to i.c. virus challenge, with LD50 values being $10^{-5.2}$ in the Balb/c mice and $10^{-4.9}$ in the Swiss Webster mice. Mean survival times varied from as early as 6.5 days, increasing as the virus challenge decreased.

These data indicated either mouse strain would be appropriate for use in the planned i.v. treatment experiments.

Conclusions

Both Balb/c and Swiss Webster mice were approximately equally susceptible to i.c. challenge with the Balliet strain of PTV; either strain was considered acceptable for anti-PTV studies.

Table XII-1. Infectivity of Baillet Strain PTV Inoculated Intracerebrally Into 4 Week-old Balb/c and Swiss Webster Mice.

Babl/c Mice

Virus Dilution	Survivors /Total	Mean Survival Time
10^{-1}	0/10	6.5
10^{-2}	0/10	7.3
10^{-3}	1/10	7.8
10^{-4}	1/10	8.8
10^{-5}	4/10	10.8
10^{-6}	9/10	10.0
10^{-7}	10/10	>21.0
10^{-8}	10/10	>21.0

LD50 = $10^{-5.2}$

Swiss Webster Mice

Virus Dilution	Survivors /Total	Mean Survival Time
10^{-1}	0/10	6.6
10^{-2}	0/10	7.2
10^{-3}	1/10	7.9
10^{-4}	1/10	8.4
10^{-5}	6/10	9.0
10^{-6}	10/10	>21.0
10^{-7}	10/10	>21.0
10^{-8}	10/10	>21.0

LD50 = $10^{-4.9}$

XIII. EFFECT OF INTRAVENOUS THERAPY ON INTRACEREBRALLY INDUCED PUNTA TORO VIRUS INFECTIONS

Introduction

To date, no agent evaluated in our program has demonstrated highly significant efficacy against the PTV infection induced by i.c. inoculation of the Balliet strain virus in mice. Investigators at Pharmatec, Inc., have developed some possible delivery systems for antiviral drugs to reach the brain, and we were asked by our COTR to work with Pharmatec in evaluating their materials. This section describes our results.

Materials and Methods

Compounds: All compounds were ribavirin (AVS01) derivatives prepared by Pharmatec, Inc., and assigned AVS numbers by Technassociates, Inc., according to the following: AVS5054, 5'-CDS-2',3-diester isobutyrate of ribavirin; AVS5055, 5'-CDS-2',3'-diester acetonide of ribavirin; AVS5056, 5'-CDS-2',3'-diester benozoate of ribavirin; and AVS5057, 5'-CDS-2',3'-diester benzoate of ribavirin. Each was considered hygroscopic and sensitive to light, air and temperature. Each was therefore stored in sealed brown bottles at -20°C until used, when used in animals, each was dissolved to the appropriate concentrations in 100% DMSO and used immediately. AVS01 (ribavirin) was provided by Technassociates, Inc. and dissolved in saline.

Virus: The Balliet strain of PTV as described earlier was used.

Animals: Female 4 week-old Swiss Webster mice weighing 19-22 g were obtained from Simonsen Laboratories for this study. All were maintained on standard mouse chow and tap water *ad libitum*, and were used after a 24 hr quarantine.

Experiment Design: As described in a separate section (XII), Balb/c and Swiss Webster mice were tested for relative sensitivities to the Balliet strain of PTV, since these mice were white, and their tail veins were more visible and i.v. injections could be more readily achieved. A preliminary titration was also done in Swiss Webster mice to determine their ability to withstand possible toxic effects of 100% DMSO administered i.v. It was found the mice could tolerate up to 0.05 ml of DMSO when injected with a 28 gauge needle attached to a 0.5 ml synnge. This volume was therefore used in the experiment with each ribavirin derivative.

An initial toxicity study was run using each ribavirin derivative injected i.v. into mice. The following doses were the maximum tolerated for each compound: AVS5054: 34.4 mg/kg; AVS5055: 175 mg/kg; AVS5056: 28.1 mg/kg; AVS5057: 25 mg/kg. At these dosages, the mice initially were prostrate immediately after injection, but then recovered within one hour and appeared normal. These doses, plus one-half, one-fourth, and one eighth of each were subsequently used in the antiviral experiments.

Two experiments were run with each compound, in the first, the compounds were injected i.v. 4 hr prior to i.c. virus inoculation. In the second experiment, the compounds were injected i.v. 24 hr post-virus inoculation. In this second experiment series, AVS01 was used as a control at dosages of 350, 175, 87.5 and 43.8 mg/kg, also administered i.v. 24 hr after i.c. virus inoculation. The mice were observed for death through 21 days.

Results and Discussion

The effects of pre-treatment with these Pharmatec materials are summarized in Tables XIII-1 through XIII-4. AVS5054 was moderately effective in prolonging life in the infected mice at the highest dose only. AVS5055 was most active of the compounds evaluated, with 40% survivors ($P<0.05$) at the highest dose used. At the MTD/4 dose, 2 of 9 infected, treated mice survived with a concomitant increased ($P<0.05$) in mean survival time. AVS5056, at the three lowest doses, caused significant ($P<0.05$) increases in mean survival time. AVS5057 was apparently ineffective, although treated, infected mice at the three highest doses survived slightly longer than placebo-treated controls.

When the experiments were repeated using delayed therapy (Tables XIII-5 to XIII-8), no significant anti-PTV effects were seen. AVS01 (Table XIII-9) was similarly ineffective when administered in its sterile saline vehicle.

These data suggest the Pharmatec derivatives administered i.v. in DMSO vehicle have potential for treating PTV-induced encephalitis in mice although it should be pointed out that

AVS01 in DMSO vehicle has not yet been evaluated in this system. Further studies should be run investigating other treatment times and possibly using multiple treatments. The i.v. treatment procedure, while somewhat difficult to perform, could be readily accomplished in the albino Swiss Webster mice used in this study.

Conclusions

Specially prepared ribavirin derivatives AVS5054, 5055, 5056 and 5057 were evaluated against i.c.-induced Balliet PTV infections in 4 week-old female Swiss Webster mice by inoculating the maximum tolerated doses (MTD), the MTD/2, MTD/4 and MTD/8 in DMSO of each i.v. 4 hr pre- or 24 hr post-virus inoculation. AVS5054 and AVS5056 pretreatment resulted in moderately significant increases in mean survival time of the infected mice. AVS5055 pre-treatment resulted in a significant increase in survivors at the MTD of the compound. AVS5057 was not effective. None of these compounds, nor AVS01 in saline, were effective when administered after virus inoculation.

Table XIII-1. Expt. PtA511. Effect of Once Only i.v. Treatment With AVS5054 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 19.6-21.7 g (4 wk) female Swiss Webster Mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.
Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5054	34.4	3/3	-1.1	0/10	9.0*[c]
	17.2	3/3	1.6	1/10	7.2
	8.6	3/3	1.5	2/10	8.4
	4.3	2/2	2.4	0/9	7.8
DMSO	-	-	-	1/18	7.8
Untreated	-	-	-	0/10	9.0
Normals	-	10/10	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]As compared to DMSO controls.

*P<0.05 **P<0.01

Conclusions: This was a Pharmatec, Inc. compound (5'-CDS-2',3'-diester isobutyrate of ribavirin) prepared per instructions from that company. Slight activity, seen as increased mean survival time, was seen at the highest dose used.

Table XIII-2. Expt. PtA512. Effect of Once Only i.v. Treatment With AVS5055 on intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 19.3-21.9 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.

Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5055	175	5/5	2.9	4/10*[c]	8.3
	87.5	4/4	1.3	0/10	7.6
	43.8	5/5	0.9	2/9	8.9*[c]
	21.9	4/4	2.1	1/10	7.9
DMSO	-	-	-	1/18	7.8
Untreated	-	-	-	0/10	9.0
Normals	-	10/10	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]As compared to DMSO controls.

*P<0.05 **P<0.01

Conclusions: This was a Pharmatec, Inc. compound (5'-CDS-2',3'-diester acetonide of ribavirin) prepared per instructions from that company. Slight activity, seen as increased mean survival time, was seen at the 43.8 mg/kg/day dose only.

Table XIII-3. Expt. PtA513. Effect of Once Only I.v. Treatment With AVS5056 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 19.3-21.9 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.
Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
	Dosage	Surv/	Host Wt.	Surv/	MST[b]
Compound	(mg/kg/day)	Total	Change (g)[a]	Total	(days)
AVS5056	28.1	5/5	1.9	0/10	8.3
	14.01	4/4	2.0	0/10	9.0*[c]
	7.0	5/5	-1.6	0/9	9.1*[c]
	3.5	4/5	1.4	0/9	9.1*[c]
DMSO	-	-	-	1/18	7.8
Untreated	-	-	-	0/10	9.0
Normals	-	10/10	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

[c]As compared to DMSO controls.

*P<0.05 **P<0.01

Conclusions: This was a Pharmatec, Inc. compound (5'-CDS-2',3'-diester pivaloate of ribavirin) prepared per instructions from that company. Slight activity, seen as increased mean survival time, was seen at three doses.

Table XIII-4. Expt. PtA514. Effect of Once Only i.v. Treatment With AVS5057 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 19.3-21.9 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.

Treatment Schedule: Once only, 4 hr pre-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5057	25	4/4	1.6	0/9	8.4
	12.5	3/3	1.1	0/10	8.0
	6.25	5/5	0.8	0/10	8.5
	3.13	5/5	0.5	0/10	7.7
DMSO	-	-	-	1/18	7.8
Untreated	-	-	-	0/10	9.0
Normals	-	10/10	-	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: This was a Pharmatec, Inc. compound (5'-CDS-2',3'-diester benzoate of ribavirin) prepared per instructions from that company. No activity was seen against PTV in this experiment.

Table XIII-5. Expt. PtA544. Effect of Once Only i.v. Treatment With AVS5054 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 19.1-20.6 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.

Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5054	34.4	5/5	-1.1	0/10	8.7
	17.2	5/5	1.6	0/9	6.7
	8.6	5/5	1.5	0/8	7.5
	4.3	5/5	2.4	0/8	7.6
DMSO	-	-	-	0/18	8.1
Untreated	-	-	-	0/20	7.5
Normals	-	5/5	2.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: 5'-CDS-2',3'-diester isobutyrate of ribavirin was ineffective when administered after i.c. PTV inoculation.

Table XIII-6. Expt. PtA545. Effect of Once Only i.v. Treatment With AVS5055 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 19.7-20.3 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5055	175	4/5	1.0	0/10	7.7
	87.5	5/5	1.2	0/10	7.3
	43.8	5/5	1.3	0/9	6.9
	21.9	5/5	1.3	0/10	7.9
DMSO	-	-	-	0/18	8.1
Untreated	-	-	-	0/20	7.5
Normals	-	5/5	2.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: 5'-CDS-2',3'-diester acetonide of ribavirin was ineffective when administered after i.c. PTV inoculation.

Table XIII-7. Expt. PtA546. Effect of Once Only i.v. Treatment With AVS5056 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 19.7-20.4 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5056	14.1	5/5	1.7	0/10	7.7
	7.0	5/5	1.7	0/10	7.2
	3.5	4/4	1.7	0/10	7.6
	1.75	5/5	1.1	0/10	6.9
DMSO	-	-	-	0/18	8.1
Untreated	-	-	-	0/20	7.5
Normals	-	5/5	2.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: 5'-CDS-2',3'-diester pivaloate of ribavirin was ineffective when administered after i.c. PTV inoculation.

Table XIII-8. Expt. PtA547. Effect of Once Only i.v. Treatment With AVS5057 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 20.3-21.4 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: DMSO.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS5057	25	5/5	1.9	0/10	8.3
	12.5	4/5	1.8	0/10	7.5
	6.25	5/5	2.4	0/10	7.9
	3.13	3/5	1.7	0/8	7.3
DMSO	-	-	-	0/18	8.1
Untreated	-	-	-	0/20	7.5
Normals	-	5/5	2.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: 5'-CDS-2',3'-diester benzoate of ribavirin was ineffective when administered after i.c. PTV inoculation.

Table XIII-9. Expt. PtA548. Effect of Once Only i.v. Treatment With AVS01 on Intracerebrally Induced Punta Toro Virus Infections in Mice.

Animals: 20.3-21.5 g (4 wk) female Swiss Webster mice.
Virus: Balliet strain Punta Toro virus, i.c. injected.
Drug Diluent: Sterile Saline.
Treatment Schedule: Once only, 24 hr post-virus inoculation.
Treatment Route: i.v.
Experiment Duration: 21 days.

		Tox. Control		Infected, Treated	
Compound	Dosage (mg/kg/day)	Surv/ Total	Host Wt. Change (g)[a]	Surv/ Total	MST[b] (days)
AVS01	350	5/5	1.4	0/10	7.5
	175	5/5	1.4	0/10	7.3
	87.5	5/5	1.2	0/10	8.7*
	43.75	5/5	1.4	0/10	7.4
Saline	-	-	-	0/20	7.3
Untreated	-	-	-	0/20	7.5
Normals	-	5/5	2.1	-	-

[a]Difference between initial weight at start of treatment and weight 18 hr following final treatment of toxicity control mice.

[b]Mean survival time of mice dying on or before day 21.

*P<0.05 **P<0.01

Conclusions: AVS01 (ribavirin) was ineffective when administered i.v. to i.c. PTV-infected mice.

XIV. INDUCTION OF LETHAL DISEASE IN MICE USING INTRANASAL ADMINISTRATION OF BALLIET STRAIN PUNTA TORO VIRUS

Introduction

It has been our experience that intracerebrally (i.c.) injected viruses produce a major challenge to antiviral drugs; if a neurotropic virus can be introduced via some route of administration other than i.c., the infection usually progresses slower and the antiviral drugs have a better opportunity to exert their viral inhibitory effects. Such an other-than-i.c. route also duplicates more closely the disease occurring in nature. We have used i.c. injection of the neurotropic Balliet strain of PTV in mice to induce the infection we are attacking with antiviral drugs. To date, few compounds have exerted acceptable antiviral effects using this model.

We have therefore attempted to induce a CNS disease in C57BL/6 mice using i.p. or intranasal (i.n.) administration of the virus. Intranasal administration of neurotropic herpesvirus type 1 has proven an acceptable means of inducing significant CNS disease in animals, and this animal model has been used successfully for antiviral experiments (1).

Materials and Methods

Virus: The Balliet strain of PTV was used in this study. The virus was diluted in MEM with 5% FBS for the study.

Mice: Three to 5 week-old C57BL/6 mice (Simonsen) were used after a 24 hr quarantine.

Experiment Design: Initially, the virus was titrated in 4 and 5 week-old mice by diluting the virus through a series of 10-fold dilutions and administering each i.p. or i.n. into 5 mice per dilution. The titration was later repeated in 3 week-old mice. Intranasal instillation was accomplished by ether-anesthetizing the mice and placing 4 drops of each virus dilution on the nares of the animal. All mice were observed for death through 21 days.

The latter i.n. titration was later repeated and an experiment was then run using the LD50 virus dose to determine if and when the virus would appear in the brains of the mice. In the study, a pool of 75 mice were infected with the virus and five mice were then killed every two days, their brains removed, and the PTV titer of each determined by assay in LLC-MK_2 cells.

Results and Discussion

The experiment was run initially in 4 and 5 week-old mice; an unsatisfactory number of animals died: 1/5 mice infected i.n., 1/5 mice infected i.p., with the highest concentration ($10^{-0.5}$ dilution) of virus. The experiment was then repeated using 3 week-old mice. With the infection via the i.p. route, the highest virus concentration ($10^{-0.5}$) again killed only 1 mouse, and this animal died by day 1 of the infection, suggesting either a toxic effect or improper injection procedure. Intranasal instillation of the virus into ether-anesthetized 3 week-old mice resulted in the deaths illustrated in Table XIV-1. These were accompanied by symptoms of central nervous system (CNS) involvement.

When the experiment was repeated, using a more concentrated virus to induce the infection, similar results were seen (Table XIV-2). The LD50 was determined to again be a $10^{-0.5}$ dilution of this virus.

The brain virus titration data are seen in Figure XIV-1. Virus was first detected on day 6, but on day 8 none was detected. On day 10, the titers reached a peak, with the mean titer being a $10^{-4.8}$ dilution of brain homogenate. The titer declined rather precipitously through the next 10 days.

Conclusions

Intranasal administration of Balliet strain PTV was lethally infective to 3 week-old C57BL/6 mice, although the LD50 was only a $10^{-0.5}$ dilution of the stock virus. The deaths occurring were accompanied by signs of CNS effects. Virus titers in the brains reached maximal levels by day 10 after initial virus exposure. This peripheral virus inoculation technique may result in a less challenging infection for evaluation of antiviral compounds. Intraperitoneal injection of PTV in either 3 or 4 week-old mice was not acceptably lethal.

References

1. Renis, N.E. 1973. Antiviral activity of cytarabine in herpesvirus-infected rats. Antimicrob. Ag. Chemother. 4:439-444.

Table XIV-1. Animal Infectivity of Balliet Strain PTV Inoculated Intranasally into 3 Week-Old C57BL/6 Mice (Initial Titration).

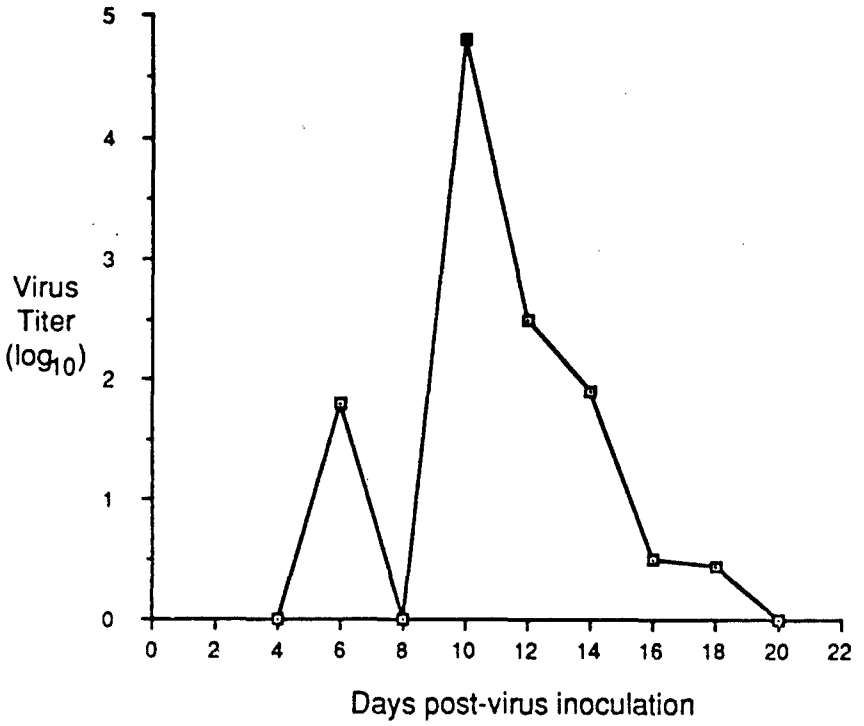
Virus Dilution	%Survivors	Mean Survival Time (days)
$10^{-0.5}$	40	14.0
$10^{-1.0}$	80	13.0
$10^{-1.5}$	100	>21.0
$10^{-2.0}$	100	>21.0
$10^{-3.0}$	100	>21.0
$10^{-4.0}$	100	>21.0

$LD50 = 10^{-0.6}$

Table XIV-2. Animal Infectivity of Baillet Strain PTV Inoculated Intranasally into 3 Week-Old C57BL/6 Mice (Follow-up Titration).

Virus Dilution	% Survivors	Mean Surv. Time (days)
Undilute	10	9.8
1:2	40	15.1
$10^{-0.5}$	50	15.4
$10^{-1.0}$	80	13.0
$10^{-1.5}$	100	>21
$10^{-2.0}$	100	>21
	LD50 = $10^{-0.5}$	

Figure XIV-1. Development of Balliet Strain PTV in Brains of Intranasally Infected Mice.



XV. PUNTA TORO VIRUS-INDUCED HEMATOLOGIC EFFECTS IN C57BL/6 MICE

Introduction

Pifat (1, 2) has described significant hematologic changes (platelet, lymphocyte, white blood cell counts) occurring in mice infected with PTV. With our recent acquisition of a Coulter EPIC-S fluorescent activated cell sorter (FACS) we felt it important to determine the hematologic effects of our particular Adames PTV stock, used in all *in vivo* chemotherapy studies, on the C57BL/6 mice used in our experiments.

Materials and Methods

Virus: Adames strain PTV as described earlier was used.

Mice: Female 3 week-old C57BL/6 mice (Simonsen) were used. They were quarantined 24 hr prior to use.

Experiment Design: Mice were infected s.c. with 0.2 ml of a 10^{-3} dilution of PTV. This was approximately a 10^2 LD50 virus inoculum. Five mice were killed prior to infection and then daily for 5 days, their blood removed with heparinized pipettes, and immediately analyzed for total white blood cells (WBC) lymphocytes, T cells and suppressor/cytotoxic T cells at each time period. In a number of cases, insufficient blood from an individual mouse was available to do all this testing; in such cases the priority was WBC > lymphocytes > T cell > suppressor/cytotoxic cells.

Results and Discussion

The results of this study are summarized in Figure XV-1. As Pifat has reported, infection with PTV results in a profound suppression of total WBC and lymphocytes. Our data also indicate T cells and suppressor/cytotoxic T cells also are suppressed at the same time. It is noted that on day 1 of the infection, the WBC and lymphocytes increased quite dramatically, probably in response to the infection. The marked suppression occurring by day 2 suggests the virus may actually attack these cells, resulting in their destruction. It may be significant that a similar, although much slower occurring observation is also seen in Acquired Immunodeficiency Syndrome (AIDS), as the virus particularly infects helper T cells.

We anticipate using reversal of hematologic change as readily determined using the FACS as an additional parameter in certain of our *in vivo* antiviral studies.

Conclusions

Infection of C57BL/6 mice with Adames PTV results in a rapid and profound suppression of WBC, lymphocytes, T cells and suppressor/cytctoxic T cells by 2 days after virus inoculation.

References

1. Pifat, D. 1985. Resistance and immunity to Punta Toro virus infections. Ph.D. Dissertation, 217 pp. Univ. of Maryland, Baltimore.
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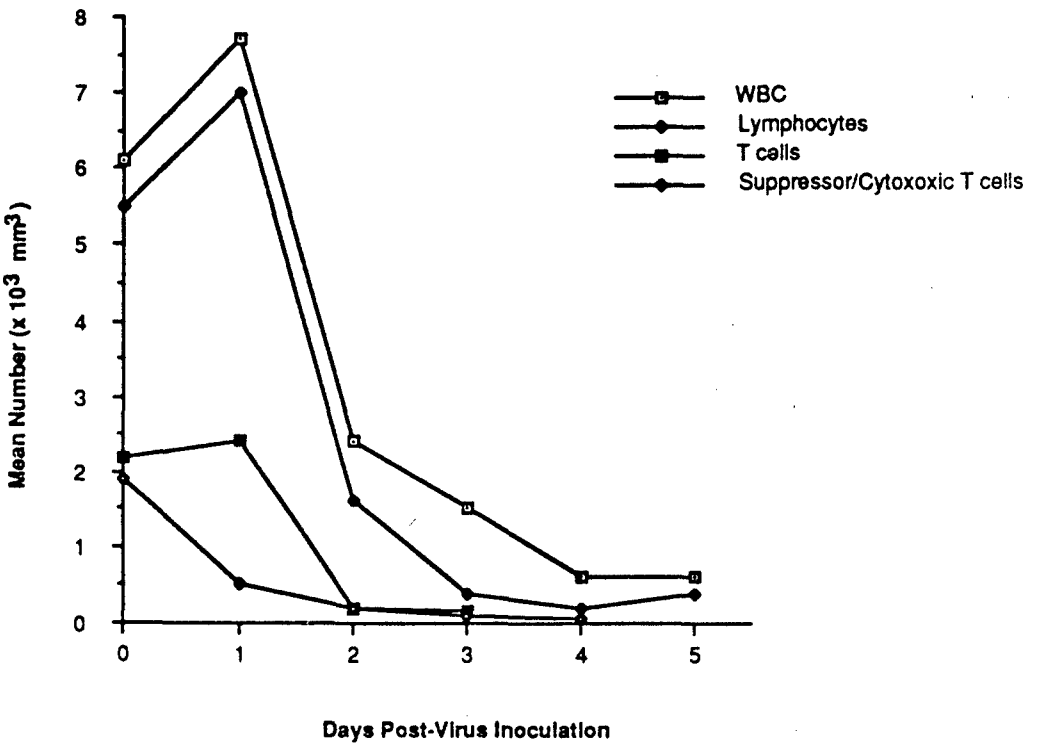
Figure XV-1. PTV-Induced Hematologic Changes in 3 Week-old C57BL/6 Mice



XVI. EFFECTS OF VARIOUS METABOLIC PRECURSORS ON THE ANTI-PUNTA TORO VIRUS ACTIVITY OF AVS206

Introduction

AVS206 (ribavirin 3-carboxamidine, ribamidine) has continued to exhibit sufficient anti-PTV activity and less *in vivo* toxicity to warrant considering this compound for eventual human use. It is therefore important to determine if it acts by a similar mechanism of action as AVS01 (ribavirin). It has previously been shown (1) that ribavirin's antiviral effects can be reversed *in vitro* by guanosine and, to a lesser extent, inosine, but not by adenosine. The carboxamidine moiety on AVS206 suggested this material may be acting as an analog of adenosine instead of guanosine. This section describes a series of *in vitro* reversal studies using AVS206 and several metabolic precursors in an attempt to further define the mechanism of anti-PTV effects of this compound.

Materials and Methods

Compounds: AVS01 and AVS206 were provided by Technassociates, Inc. All metabolic precursors used in the reversal experiments were purchased from Sigma Chemical Co. (St. Louis, MO).

Virus: The Adames strain of PTV as previously described was used in these studies.

Cells: LLC-MK_2 cells as described previously were used.

Experiment Design: All *in vitro* PTV experiments were performed as described earlier for our routine anti-PTV evaluations, except as noted below.

Initially, the plaque-inhibitory effects of 200, 100, and 50 µg/ml of AVS206 were determined in the presence and absence of 200 µg/ml of adenosine, inosine and guanosine.

In a second series of reversal studies, the effects of adenine, adenosine, cytidine, guanosine, guanosine-5'-PO_4, inosine, thymidine, uridine, and xanthosine were evaluated on PTV *in vitro* and on the anti-PTV effects of AVS206 *in vitro*. In each experiment, 200 µg/ml of the metabolic precursor was used against 1000, 320, 100, 32, 10 and 3.2 µg/ml of AVS206. In this study, inhibition of viral CPE using our standard 96-well microplate system was employed.

A third reversal experiment was run using other related compounds, these being 2'-deoxyadenosine, 2'-deoxycytidine, 2'-deoxyguanosine, hyproxanthine, inosine-5'-PO_4 and orotodine. These compounds were used because previously we also used these materials in reversal studies with ribavirin using other viruses. This third study also used CPE inhibition in 96-well microplates as infectious parameter.

A single reversal study has been run to date using AVS01 (ribavirin) against PTV. Inhibition of CPE was used in this study, with only guanosine and xanthosine used as reversal agents, since these metabolic precursors have previously been found to reverse ribavirin's anti-measles and herpesvirus effects.

Results and Discussion

The plaque-inhibitory effects of 200, 100 and 50 µg/ml of AVS206 were determined in the presence and absence of 200 µg/ml of adenosine, inosine and guanosine. The results of this experiment are seen in Table XVI-1. The anti-PTV activity of AVS206 were essentially completely reversed by guanosine. Inosine had no effect. A moderate reversal was seen with adenosine, but an unusual observation was made that adenosine alone, at 200 µg/ml, was inhibitory to PTV-induced plaques.

The results of the second reversal experiment are seen in Table XVI-2. AVS206 was completely inhibitory to PTV CPE at 1000, 320 and 100 µg/ml; slight CPE was seen at 32 µg/ml. No inhibitory effect was seen at lower dosage levels. Definite reversal of the antiviral activity of AVS206 was seen using adenine, adenosine, guanosine, guanosine-5'-PO_4, and inosine. All of the materials used appear to have a slight reversal on AVS206 antiviral activity at the 320 µg/ml dosage of AVS206; we felt this effect may have been non-specific, so considered only those materials that allowed viral CPE to be seen at 100 µg or higher dose levels of AVS206. It is interesting to note that adenosine, and to a lesser extent, adenine, exhibited a PTV-CPE inhibitory effect when used alone. Both materials also exerted a slight effect on uninfected cells, however; we therefore attribute the antiviral effects of these compounds to a non-specific toxic or static effect.

The results of use of other metabolic precursors as reversing agents with AVS206 are summarized in Table XVI-3. 2-Deoxyadenosine and 2'-deoxyguanosine were considered reversing agents in this experiment.

The results of the reversal study with AVS01 are seen in Table XVI-4. Only guanosine and xanthosine were run in this initial study. Guanosine exerted a definite reversal effect, as has been reported previously (1).

At present, considering past reversal experiments run with ribavirin using other RNA viruses, these data suggest that AVS206 may have a similar mechanism of action as ribavirin; i.e., acting as an analogue of guanosine and inhibiting guanosine monophosphate biosynthesis in the infected cell. Ribavirin has previously not been shown to be reversed by adenine, adenosine or 2'-deoxyadenosine; AVS206 does seem to be reversed by these materials. This suggests to us that the compound is also acting as an analogue of adenosine in the cell. It is possible that AVS206 is being partially metabolized to ribavirin in the cell, thus exerting its antiviral effect as both compounds. Our ongoing comparative reversal studies with ribavirin should further determine if this postulate is correct.

Conclusions

The *in vitro* anti-PTV activity of AVS206 was reversed by adenosine, 2-deoxyadenosine, guanosine, guanosine 5'-PO_4, and 2-deoxyguanosine. Other precursors, including inosine, adenine, cytidine, thymidine, uridine, and xanthosine did not have a noticeable reversal effect. The anti-PTV activity of AVS01 was reversed by guanosine but not by xanthosine.

References

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Table XVI-1. PTC666-669. Reversal of AVS-206 Punta Toro Virus Plaque Inhibition by Adenosine, Guanosine, or Inosine[a].

Concentration AVS206 (µg/ml)	Avg. No. Plaques/Well			
	No Additive	Adenosine (200 µg/ml)	Inosine (200 µg/ml)	Guanosine (200 µg/ml)
200	0	0	0	48
100	0	17	0	58
50	35	12	42	68
0	68	0	74	76

[a]Plaques induced in LLC-MK_2 cells. Metabolites added concomitantly with AVS206 to infected cells.

Table XVI-2. PTC641-650. Reversal of AVS-206 Anti-Punta Toro Virus Activity by Various Purines, Nucleosides or Nucleotides[a].

Concentration AVS-206 (µg/ml)	Average CPE Score[b] @ Additive Concentration Shown									
	No Additive	Adenine (200 µg/ml)*	Adenosine (200 µg/ml)*	Cytidine (200 µg/ml)	Guanosine (200 µg/ml)	Guanosine 5'-PO_4 (200 µg/ml)	Inosine (200 µg/ml)	Thymidine (200 µg/ml)	Uridine (200 µg/ml)	Xanthosine (200 µg/ml)
1000*	0	0	0	0	0	0	0	0	0	0
320*	0	0	0.2	0	0.5	0.5	0	0	0	0
100*	0	0.5	1.0	0	2.0	2.7	0.3	0	0	0
32	0.7	1.7	3.2	1.3	3.5	4.0	4.0	3.5	3.7	2.0
10	4.0	3.0	1.3	4.0	4.0	3.8	4.0	4.0	4.0	4.0
3.2	4.0	2.7	1.2	4.0	4.0	4.0	4.0	4.0	4.0	4.0
0	4.0	2.7	0.7	4.0	4.0	4.0	4.0	4.0	4.0	4.0

[a]CPE induced in LLC-MK_2 cells. Each compound added concomitantly.

[b]CPE scored from 0 (normal cells) to 4 (maximal cell destruction).

*Slight cytotoxicity also seen using these materials.

Table XVI-3. PTC670-676. Further Studies on the Reversal of AVS-206 Anti-Punta Toro Virus Activity by Various Compounds[a].

Concentraion AVS-206 (µg/ml)	Average CPE Score[b] @ Additive Concentration Shown						
	No Additive	2'-Deoxy-adenosine (200 µg/ml)*	2'-Deoxy-cytidine•HCl (200 µg/ml)	2'-Deoxy-guanosine (200 µg/ml)	Hypoxan-thine (200 µg/ml)	Inosine 5'-phosphate Na salt (200 µg/ml)	Orotidine (200 µg/ml)
1000*	0	0	0	0	0	0	0
320*	0	0.2	0	0.5	0	0	0
100*	0	1.8	0.3	2.7	0.3	0.3	0.3
32*	2.8	4.0	3.7	3.8	3.8	4.0	3.5
10	4.0	4.0	4.0	4.0	4.0	4.0	4.0
3.2	4.0	4.0	4.0	4.0	4.0	4.0	4.0
0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

[a]CPE induced in LLC-MK_2 cells. Each compound added concomitantly.

[b]CPE scored from 0 (normal cells) to 4 (maximal cell destruction).

*Slight cytotoxicity also seen using these materials.

Table XVI-4. PTC651-653. Reversal of AVS-01 Antiviral Activity by Guanosine or Xanthosine[a].

Concentration AVS-01 (µg/ml)	Average CPE Score[b] @ Additive Concentration Shown		
	No Additive	Guanosine (200 µg/ml)	Xanthosine (200 µg/ml)
1000	0	0	0
320	0	0	0
100	0	0.5	0
32	0.5	2.3	0
10	2.0	3.7	0.8
3.2	3.5	3.8	4.0
0	4.0	4.0	4.0

[a]CPE induced in LLC-MK_2 cells. Each compound added concomitantly.

[b]CPE scored from 0 (normal cells) to 4 (maximal cell destruction).

XVII. EFFECTS OF AVS01 AND AVS206 ON UPTAKE OF RADIOLABELED METABOLIC PRECURSORS AS A MEASURE OF CYTOTOXICITY

Introduction

To further elucidate possible cytotoxic or cytostatic effects of AVS01 (ribavirin) and AVS206 (ribavirin 3-carboxamidine, ribamidine), the effects of these compounds on cellular DNA, RNA and protein synthesis were determined.

These effects were ascertained by measurement of inhibition of uptake of [^{3}H]thymidine and inorganic ^{32}P for DNA effects, [^{3}H]uridine for RNA synthesis effects, and [^{3}H]leucine for protein synthesis.

Materials and Methods

Cells: Rhesus monkey kidney (LLC-MK$_2$) cells as previously described were used.

Compounds: AVS01 and AVS206 were provided by Technassociates, Inc. Each was dissolved in MEM with $NaHCO_3$ and gentamicin for these studies. All radiolabeled materials were obtained from ICN Pharmaceuticals, Inc. (Irvine, Calif.).

Experiment Design: Experiments were run in 96 well disposable microplates using 4 wells for each drug concentration and 8 wells for the drug-free control. All drugs and controls were run on the same plate for each radiolabeled precursor.

Approximately 1.5 x 10^5 cells/well in a 24 hr monolayer were incubated with each drug concentration for 3 hr at 37°C followed by a cell wash and a 1 hr pulse with 10 µCi/ml radiolabel in the presence of drug. Fetal bovine serum was absent during the entire period of drug treatment and pulse. Following the pulse period, the medium was aspirated from the cells and 10% sodium dodecyl sulfate (0.1 ml/well) was added. After a 5 min. mild shaking period, 0.1 ml of 20% trichloroacetic acid (TCA) was added and the plates were incubated at 40°C for 2 hr to affect complete precipitation. The precipitate was filtered onto 0.45 µm Millipore filters using 5% TCA for rinse. The dried filters containing the radiolabeled precipitate were then counted on a Packard Scintillation Counter. Percentages of drug-free controls were determined in each dose. These procedures were similar to those described previously by us (1) and by others (2-4).

Results and Discussion

The results of these studies are summarized in Tables XVII-1-4. Using [^{3}H]thymidine incorporation, neither ribavirin nor ribamidine were demonstrably inhibitory to cellular DNA synthesis. Using ^{32}P incorporation, ribavirin was inhibitory to DNA synthesis at all concentrations used. AVS206 was inhibitory only at 1000 µg/ml. [^{3}H]Uridine incorporation (RNA synthesis) was not significantly inhibited by any dose of ribavirin, and only by the 1000 and 100 µg/ml dosages of the carboxamidine. These ribavirin data showing an effect on RNA synthesis do not match previous data we have reported with this compound using MA-104 cells (1) and may be due to the cell differences. Such a variation in ribavirin's effects due to cell differences have been reported previously (5). Both compounds were considered to have moderate effects on protein synthesis (Table XVII-4), although these effects were not dose-responsive.

The data with ribavirin regarding inhibition of protein synthesis correlate well with those previously reported (1). This latter effect may be a general effect on amino acid transport into the cell, rather than direct inhibition of protein synthesis. These observations suggest both compounds to have a static, rather than a toxic effect on the cell. Of importance in these assays was the use of an established and confluent cell monolayer in contrast to rapidly dividing cells often used in such studies (2, 3). Such cell monolayers, exposed for a relatively long period of time to the test compounds prior to pulsing, were used to more closely duplicate the conditions of the antiviral experiments.

Conclusions

Neither AVS206 nor AVS01 were considered strongly cytotoxic as measured by effects on DNA, RNA, and protein synthesis. AVS206 was less inhibitory to DNA synthesis as measured by uptake of ^{32}P than AVS01. Our data suggest both materials to have a static, rather than toxic, effect on LLC-MK$_2$ cells.

References

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Table XVII-1. Effects of AVS01 and AVS206 on ^{3}H Thymidine Incorporation into LLC-MK$_2$ Cells[a]

Compound	Drug Concentration (µg/ml)	^{3}H Thymidine Incorporation CPM[b]	% of Control ± SE[c]
AVS01	1000	24,993	105 ± 20
	100	21,886	91 ± 5
	10	22,884	95 ± 9
	1	27,434	115 ± 7
	0.1	27,434	115 ± 4
	0	23,887	100 ± 5
AVS206	1000	22,535	90 ± 18
	100	24,522	102 ± 10
	10	32,617	137 ± 5
	1	31,056	130 ± 10
	0.1	31,753	133 ± 8
	0	23,887	100 ± 5

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

Table XVII-2. Effects of AVS01 and AVS206 on Inorganic ^{32}P Incorporation into LLC-MK_2 Cells[a]

Compound	Drug Concentration (µg/ml)	^{32}P Incorporation CPM[b]	^{32}P Incorporation % of Control ± SE[c]
AVS01	1000	2,955	50 ± 7
	100	2,205	37 ± 6
	10	3,643	59 ± 4
	1	3,498	62 ± 8
	0.1	3,754	63 ± 5
	0	5,924	100 ± 16
AVS206	1000	3,677	62 ± 10
	100	6,486	110 ± 3
	10	6,199	104 ± 3
	1	7,317	124 ± 2
	0.1	10,888	184 ± 6
	0	5,924	100 ± 16

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

Table XVII-3. Effects of AVS01 and AVS206 on ^{3}H-Uridine Incorporation in LLC-MK$_2$ Cells[a]

Compound	Drug Concentration (µg/ml)	^{3}H Uridine Incorporation CPM[a]	^{3}H Uridine Incorporation % of Control ± SE[b]
AVS01	1000	113,888	105 ± 7
	100	99,257	89 ± 6
	10	98,207	88 ± 6
	1	137,961	125 ± 8
	0.1	128,426	116 ± 15
	0	110,617	100 ± 7
AVS206	1000	49,489	44 ± 8
	100	46,552	42 ± 5
	10	130,636	116 ± 2
	1	121,897	102 ± 7
	0.1	127,403	115 ± 5
	0	110,617	100 ± 7

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

Table XVII-4. Effects of AVS01 and AVS206 on ^{3}H-Leucine Incorporation into LLC-MK$_2$ Cells[a]

Compound	Drug Concentration (µg/ml)	^{3}H Leucine Incorporation CPM[b]	% of Control ± SE[c]
AVS01	1000	91,223	52 ± 6
	100	113,893	64 ± 2
	10	131,734	75 ± 4
	1	116,979	67 ± 5
	0.1	131,497	75 ± 4
	0	175,252	100 ± 7
AVS206	1000	77,394	44 ± 4
	100	113,340	65 ± 10
	10	107,411	61 ± 12
	1	112,634	64 ± 12
	0.1	119,139	68 ± 10
	0	175,252	100 ± 7

[a]24 hr monolayer initially, then incubated with drug for 23 hr, followed by 1 hr radiolabelled pulse.

[b]Mean of four replicates.

[c]Standard Error of percentage.

XVIII. INVESTIGATIONS INTO THE DEAMINATION OF AVS206

Introduction

Compounds AVS01 (ribavirin) and AVS206 (ribavirin 3-carboxamidine, ribamidine) are almost identical, with the exception that the carboxamide group of AVS01 is converted to a carboxamidine group to give AVS206. AVS206 is thought to be merely deaminated to AVS01 *in vitro* and *in vivo*, presumably *in vivo* by host deaminases. In addition, AVS206 in aqueous solution is also known to hydrolyze to AVS01, especially upon prolonged storage (R.K. Robins, ICN Pharmaceuticals, *personal communication*). This study will provide evidence that AVS206, after being treated with adenosine deaminase, is altered to a compound that comigrates with AVS01 on silica gel thin lay chromatography plates.

Materials and Methods

Materials: Inosine, guanosine, adenosine, and adenosine deaminase (type VI) were obtained from Sigma, St. Louis, Missouri. Fluorescent silica gel plates (LKGDF, 5 x 20 cm) were purchased from Whatman (Hillsboro, Or).

Deamination: Nucleosides and analogs were suspended in 10 mM phosphate buffer, pH 6.5. Each compound was incubated with enzyme (50:1, compound to enzyme) at 25° C for 1 hour.

Development and Detection: Five microliter samples were directly applied to silica gel plates without stopping the deaminase reaction and dried at room temperature for 15 minutes. Plates were developed with 2-propanol-aqueous ammonia (fresh)-H_2O (7:1:2). Using ascending chromatography, plates were developed for about 3 hours until solvent front migrated 15 cm. Plates were allowed to air dry overnight to eliminate any traces of free ammonia.

Plates were placed in a 2000 ml beaker layered with a 0.5 cm layer of $CaHClO_3$, covered, and incubated for 2 min. The plates were then transferred to another 2000 ml beaker and placed down in the large beaker beside a smaller beaker filled with 20 ml of formalin. The larger beaker was covered and the plates were exposed to the formalin for 45 seconds. The plates were removed and immediately sprayed with a fine mist of 1% soluble potato starch, 1% potassium iodide, and 0.05% Triton X-100 (freshly made). The N-chlorinated nucleosides or analogs were detected as dark purplish-black spots. Migration distances of each compound were measured relative to the solvent front in each track.

Results and Discussion

A variety of compounds can be deaminated by adenosine deaminase, including adenosine, guanosine, 2,6-diaminopurine(2'-deoxy)riboside, and 6-methoxypurine. Therefore, adenosine deaminase was chosen as the likely enzyme that could deaminate AVS206, because of the relative lack of specificity of the enzyme.

When treated with adenosine deaminase, the migration of AVS01 was unaffected and adenosine, as expected, was converted to inosine as well as other compounds (Table XVIII-1, Figure XVIII-1). Guanosine did not deaminate. However, AVS206 was converted to two migrating species, one that comigrated with AVS01 and another species that presented itself as a broad, smeared, band. The species with an R_f value of 71 may have been ribavirin. The smeared band probably represents several compounds including AVS206. Some of these compounds could be stable enzymatically-derived intermediates or perhaps a final end product such as an NH_2OH derivative. Further support for this hypothesis is the fact that when AVS206 was stored for a prolonged time period and consequently hydrolyzed non-enzymatically, only two discrete bands appeared after chromatography (Figure XVIII-1). Smeared bands only appeared when the substrate was susceptible to deaminase cleavage. Contaminants due to buffer or water were also not contributors to the smearing (Table XVIII-1). Smearing due to sample overload can also be eliminated as a factor, since preparations without enzyme did not smear as seen in Table XVIII-1. Contaminating enzymes in the deaminase preparation which could also generate different species of compounds were not important due to their low concentration in the enzyme preparation (5'-AMP deaminase,<0.002%; alkaline phosphotase,0.004%; guanase, <0.001%; nucleoside phosphorylase, 0.005%; percentage of adenosine deaminase activity).

Since adenosine deaminase is a common serum deaminase and also a major contaminant of fetal bovine serum, AVS206 could very well be deaminated in *in vitro* and *in vivo* to AVS01 by this enzyme. But, this does not explain the different efficacies of AVS206 and AVS01 against PTV *in vivo* as we have reported (1). However, the possibility exists that some stable intermediate form of AVS206 (perhaps a species found in the smeared band R_f=55-65) may be generated by deaminase activity that could provide the striking anti-PTV activity of AVS206. It is also possible that the compound is acting as a prodrug for ribavirin.

In a separate experiment, AVS206, dissolved in MEM supplemented with 0.1% $NaHCO_3$, was stored at 5°C for 2 weeks, after which it was evaluated as above for chromatographic migration. A definite separation of a portion of the compound to AVS01 was seen (Table XVIII-1), indicating that the compound does indeed hydrolyze to a compound which we presume to be ribavirin upon prolonged storage.

Conclusions

AVS206 and AVS01 were subjected to enzymatic degradation with adenosine deaminase. AVS206 was broken down to a species that comigrated with AVS01 as determined by silica gel thin layer chromatography. AVS01 was unaffected by deaminase treatment. In addition, with prolonged incubation, AVS206 apparently degenerates to a species that also comigrates with AVS01.

References

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Table XVIII-1. The Effects of Deaminase Treatment of Selected Nucleosides and Nucleoside Analogs.

Compound	Relative Migration Distance (R_f)[a]	
	Untreated	Deaminase Treated
AVS 01	72	72
AVS 206	63,72[b]	71,55-65[bc]
Adenosine	75	60-70[c]
Inosine	65	65
Guanosine	75	75
Buffer[d]	75	75
AVS 206[e]	63,72[b]	ND

[a]All Distances were measured relative to solvent front using a template spotting guide.

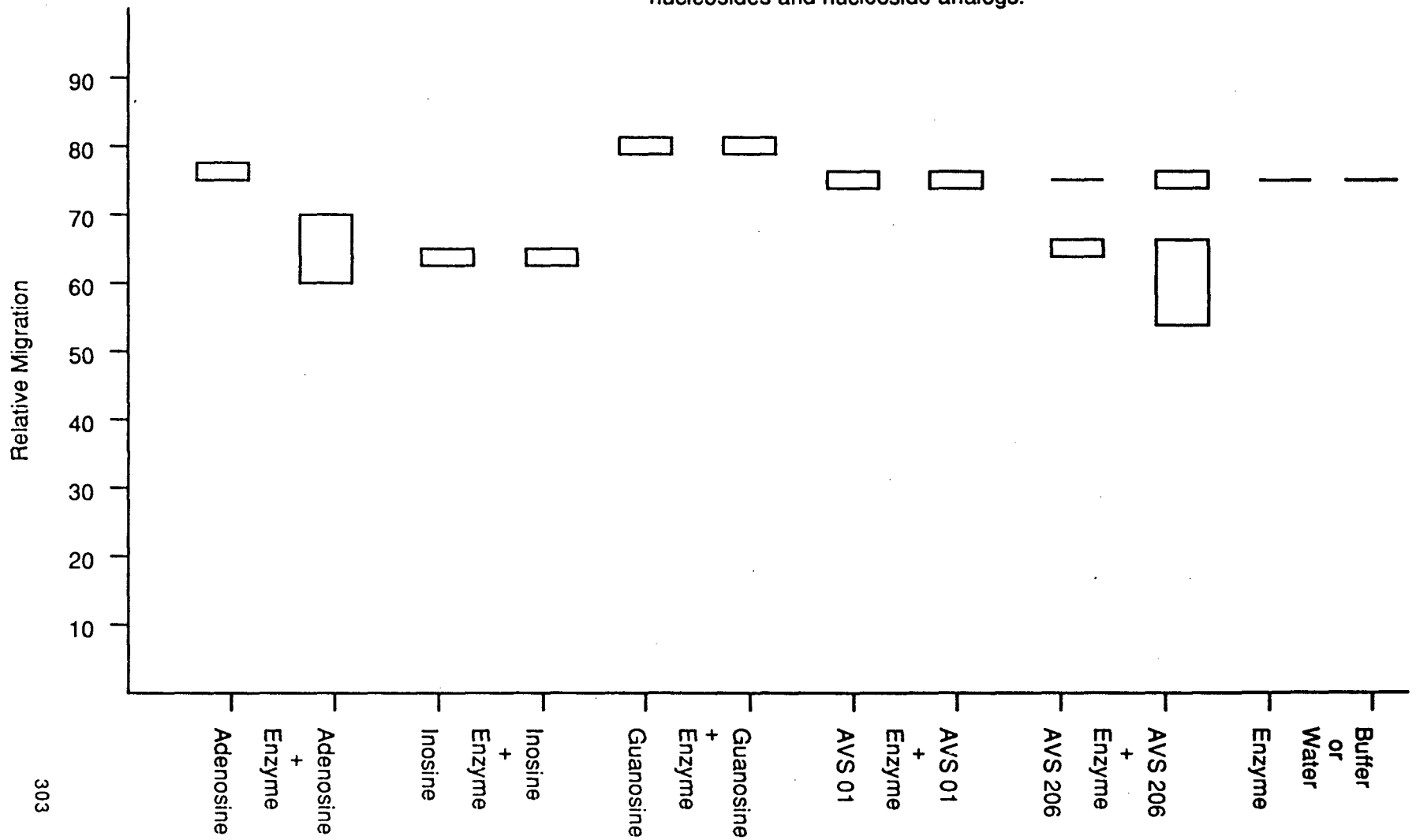
[b]Minor band.

[c]Band was smeared

[d]Buffer with or without enzyme, no nucleoside added.

[e]AVS 206 was incubated in MEM supplemented with 0.1% sodium bicarbonate at 5°C for 2 weeks and then sampled.

Figure XVIII-1. The effects of deaminase treatment of selected nucleosides and nucleoside analogs.



XIX. INTERFERON INDUCTION BY AVS2776

Introduction

One of the most significant anti-PTV compounds evaluated to date has been AVS2776 (bropirimine, ABPP, 2-amino-5-bromo-6-phenyl-4(3H)pyrimidinone), which is an immunomodulating agent. This study was run to determine the time of interferon (IFN) induction by this compound in the 3 week-old C57BL/6 mice used in our PTV studies.

Materials and Methods

Compound: AVS2776, a product of UpJohn Co., was submitted to us via Technassociates, Inc. The compound was prepared in 0.4% carboxymethylcellulose (CMC) in stoppered bottles and stored overnight at room temperature until used.

Interferon Assay: Samples to be assayed for IFN were placed in 0.1 ml volume on an 18-24 hr monolayer of L cells in 96 well microplates and allowed to incubate for 24 hr at 37°C. After incubation, the cells were drained and 0.1 ml of a 10^3 CCID50/0.1 ml of vesicular stomatitis virus (VSV) strain Indiana was added to each and incubated for 6 days at 37°C. Viral CPE was read microscopically after this incubation. The IFN titer was expressed as units/0.1 ml based on the maximum dilution of serum sample that inhibited VSV CPE by 50% or greater. No attempt was made to separate out IFN α, β, or γ, so we must presume all types were present in the samples assayed. Controls in the study were virus controls, which were cells exposed to test medium (MEM with 2% FBS, 0.18% $NaHCO_3$ and 50 µg gentamicin/ml) and then to VSV, and cell controls which were exposed to test medium only.

Animals: Three week-old male C57BL/6 mice obtained from Simonsen Laboratories were used. They were caged 10 to a cage and fed Wayne Lab Blox mouse chow and tap water *ad libitum*.

Experiment Design: Groups of 35 mice each were treated a single time p.o. with AVS2776 in dosages of 50, 100, 200 or 400 mg/kg. Five animals were killed and their blood taken 2, 4, 6, and 8 hr later. Five normal mice were also killed and bled at the 2 hr time periods to provide baseline data. Serum was separated from each sample frozen at -70°C and later assayed individually for IFN titer.

Results and Discussion

The results of this study are summarized in Table XIX-1. Maximal IFN titers were seen at the initial, 2 hr time of sampling; by 8 hr the titers had declined to near undetectable levels. Surprisingly, the 100 mg/kg dosage of AVS2776 was most effective in stimulating IFN, yet the 50 mg/kg dosage was almost not effective. Considered variation was seen as evidenced by the wide standard errors (S.E.) seen in the table. These were due primarily to one or two mice in a group that had abnormally high IFN levels. This compound is quite insoluble in the aqueous vehicle used, so it is very possible that one animal could have received a larger dose of the drug than another, explaining the possible wide variations seen. In addition, the p.o. (gavage) route of administration may not always deliver an exact quantity of drug to each mouse, which may be another reason for the variation seen..

This very rapid induction of IFN could explain why AVS2776 is so effective when given as late as 48 hr after PTV exposure as we have described in earlier reports. Since the 50 mg/kg dosage of AVS2776 was minimally effective in inducing IFN, it also explains why this dosage of the compound is only marginally effective against the PTV infection.

It is most significant that this compound is capable of inducing large amounts of IFN when administered by the oral route, since no other immunomodulators that we have to date evaluated, except chemical analogs of this compound, are effective orally.

Conclusions

AVS2776 was shown to be a potent and rapid inducer of interferon (IFN) at doses of 100-400 mg/kg given in a single oral gavage treatment to 3 week-old C57BL/6 mice. The IFN levels were maximal at 2 hr after treatment. A dose of 50 mg/kg did not induce acceptable levels of IFN.

Table XIX-1. Serum IFN in 3 Week-Old C57BL/6 Mice Treated with AVS2776.

AVS2776 Dosage (mg/kg)	Mean IFN Titer (units/0.1 ml) ± S.E.			
	2 hr	4 hr	6 hr	8 hr
400	2686±3607	1158±1187	1605±2850	135±98
200	2724±2227	1067±971	620±650	40±30
100	14,953±13,873	1272±1036	15±16	0
50	88±31	26±16	29±32	0
0	0			

XX. IMMUNOLOGICAL EFFECTS OF AVS2149 IN THREE-WEEK-OLD C57BL/6 MICE

Introduction

We have previously reported that AVS2149 (ampligen, poly I•poly C12u) is markedly effective when used against Adames PTV infections in mice. It is known that this material is a potent interferon (IFN) inducer (1), an inducer of high levels of splenic natural killer cell activity and a good activator of peritoneal macrophages (2). We considered it important to determine other immunological effects of this material, particularly in the immature 3 week-old C57BL/6 mice used in our PTV experiments, so as to better determine the most appropriate treatment regimen for this material and to more fully ascertain how much promise this compound will have for possible human use.

Materials and Methods

Animals: Three week-old female C57BL/6 mice obtained from Simonsen Laboratories were used. They were submitted to a 24 hr quarantine prior to use in these studies.

Compound: AVS2149 was obtained from Technassociates, Inc. for use in these studies. The compound was provided in dry form in sealed vials. This was annealed by adding 20 ml of sterile pyrogen-free water to a vial, which was then placed in a 65°C water bath for 30-40 minutes, then allowed to sit at room temperature for 1 hr. The contents were then refrigerated until used. The contents were diluted in sterile phosphate-buffered saline (PBS) for use in these studies.

Macrophage Function Test: Interleukin-1 (IL-1) production was assayed as a measure of macrophage function. The basis of this method is to first stimulate macrophages with lipopolysaccharide (LPS) to produce IL-1 whose activity is then assayed by its ability to stimulate the proliferation of target cells. We used human astrocytoma cells (U373 cell line from ATCC) as the target cells according to the report of Lachman et al. (3).

One ml suspension of splenic cells (5 x 10^6 cells) in RPMI-20% FCS was pipetted into wells of a 24-well microcultureplate. Macrophages, by virtue of their property of adherence to plastic surface, were allowed to adhere to the plastic surface. After 1 hr, the non-adherent cells were removed, and fresh growth medium containing LPS (20 μg/ml) was added. The plates were then placed inside the humified chamber of a CO_2 incubator at 37°C for 24 hours. Afterwards, the contents were collected with a pasteur pipette and centrifuged to collect the IL-1 containing supernatant. For the assay of IL-1 activity, the confluent monolayers of U373 cells were freshly trypsinized, 10,000 cells in 0.1 ml were pipetted into each well, allowed to recover for 4-6 hr, and then 0.1 ml of either control or test samples of IL-1 containing supernatant was added. We usually test 4 dilutions: undiluted, 1:2, 1:4, and 1:8. As a positive reference, we also set up 6 concentrations of recombinant human rIL-1 (Cistron Tech., NY). After 24 hr of treatment, the cells were pulse-labelled with 0.5 μCi of [^{3}H]thymidine for an additional 16 hr, they were harvested, and processed for the counting of radioactivity in the same fashion as described below for the lymphocyte blastogenic test. At the end, the IL-1 response was obtained in terms of the counts/min of thymidine uptake (test - control counts/min). The data are given as the maximum response.

Cell Enumeration Assay: Spleen and thymic lymphocytes were enumerated with monoclonal antibodies using a Coulter Epic C fluorescence-activated cell sorter (FACS). The cells were enumerated by incubating them with each of the following fluorescence-tagged monoclonal antibodies: Anti-Thy 1.2 (T cells), anti-mouse Ig (B-cells), anti-L3T4 (helper T cells) and anti-Lyt2 (suppressor/cytotoxic T cells). The proportion of the cells stained positive with various monoclonal antibodies were then quantitated by the standard procedure of flow cytometric analysis.

T Cell Function Test: This was assessed in the blastogenic response to stimulation by mitogens: Phytohemagglutinin (PHA) or concanavalin A (con A). The splenic cells were cultured for 3 days in the absence or presence of PHA (2%) or con A (10 ≤g/ml). Afterward, the cells were pulse-labeled with 0.5 ≤Ci of [^{3}H]thymidine for an additional 16 hr. The cells were harvested using a cell harvester (Flow Laboratories) and the glass-fiber paper discs counted in 2 ml of scintillant. The blastogenic response is given in terms of mean counts per min of triplicates. The growth

medium used was RPMI-1640 containing 10% fetal calf serum (FCS) and streptomycin-penicillin mixture.

B Cell Function Test: The blastogenic response of spleen cells to a B-cell mitogen, known as *E. coli* lipopolysaccharide (LPS) was measured. A total of 100,000 splenic cells in a 0.1 ml volume of 10% FCS in RPMI-1640 was added to triplicate wells of 96-well flat-bottomed microplates. LPS (50µg/ml) in 0.1 ml was added to the wells. The plates were then incubated for 72 hr at 37°C and the cells were pulsed, harvested and counted in the same fashion as above for the T cell function test.

Experiment Design: Groups of 6 mice each were treated i.p. with 1, 3.2 or 10 mg/kg/day of AVS2149 or with sterile placebo (PBS). After holding the animals 24 hrs, all were killed and their spleens and thymuses removed aseptically and placed in tubes containing cold RPMI-1640 medium. Each was teased apart and the individual spleen and thymic cells separated via passage through a wire mesh, then submitted for enumeration o T, B, T helper and T suppressor cells, for B and T cell lymphocyte function tests, and for macrophage function testing.

Results and Discussion

The percentage distributions of T, B, T_H and T_S cells from the spleen and thymus of mice treated with AVS2149 are seen in Table XX-1. This compound appeared to significantly increase splenic B cell distribution at all dosages; the highest dosage (10 mg/kg) also resulted in a 2-fold increase in splenic T_S cells. In the thymus, T cells were increased significantly in mice treated with all dosages of this compound, yet the relative proportions of T_H or T_S cells were not altered, and B cells were undetectable as expected.

The lymphocyte function test results are summarized in Table XX-2. It appeared that despite T and B cell distribution, their function was significantly reduced in AVS2149-treated mice. We, however, noted a considerable unexplained variation in some of the values obtained, so these results should be interpreted with caution.

In addition, we found a dose-responsive suppression of IL-1 activity (macrophage function) in mice treated with AVS2149 (Table XX-3).

These data suggest this IFN inducer to have a range of immunological effects, some being potentially of an adverse nature, perhaps an immunosuppressive. To our knowledge, these tests have never been run with ampligen by other investigators.

Conclusions

Single i.p. treatment of 3 week-old C57BL/6 mice with 1, 3.2, or 10 mg/kg of AVS2149 resulted in an increase in splenic B cells and T_S cells and an increase in T cells in the thymus. B and T cell function and macrophage function (IL-1 activity) appeared to decrease in these treated animals.

References

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Table XX-1. Expt. PT-139. Effect of AVS2149 Treatment on Percentage Distribution of T Cells, B Cells, T Helper (T_H) Cells and T Suppressor (T_S) Cells in the Spleen and Thymus of C57BL/6 Mice.

AVS2149 Dosage (mg/kg)	%Distribution of Cells in the Spleen and Thymus							
	Spleen				Thymus			
	T	B	T_H	T_S	T	B	T_H	T_S
10	23.8	44.2**	10.4	21.2**	79.8**	0	73.2	93.8
3.2	18.0	44.7**	5.8	10.2	76.2**	0	72.3	92.8
1	17.4	34.2	7.8	11.3	74.0**	0	75.2	92.2
0	20.0	30.5	9.2	10.3	54.8	0	74.7	90.5

**P<0.01

Table XX-2. Expt. PT-139. Effect of AVS2149 Treatment on Lymphocyte Function[a] in C57BL/6 Mice.

AVS2149 Dosage (mg/kg)	Mean Counts Per Minute/Stimulation Index[b] PHA (2%)	Con A (10 µg/ml)	LPS (50 µg/ml)
10	505/1.6	1,618/5.3	16,198/53
3.2	366/1.0	420*/1.2	19,434/56
1	562/2.1	1,875/7.2	12,526*/48
0	404/2.0	3,178/16.0	23,452/115

[a]Mitogen-induced stimulation of splenic cells (^{3}H-thymidine uptake into DNA).

[b]Stimulation Index = (cpm with mitogens)/(cpm background)

*$P<0.05$

Table XX-3. Expt. PT-139. Effect of AVS2149 Treatment on Macrophage Function[a] in C57L/6 Mice.

AVS2149 Dosage (mg/kg)	Mean Counts/ Minute	% of Control
10	522	58%
3.2	596	62%
1	752	84%
0	900	

[a]Interleukin-1 activity

XXI. EFFECTS OF DILUENT ON *IN VITRO* AND *IN VIVO* INFECTIVITY OF PUNTA TORO VIRUS

Introduction

Despite careful titrations of stock PTV prior to use in our *in vivo* antiviral experiments, we have continually encountered situations where a virus dilution known to be 100% lethal in titrations has not been acceptably lethal to the mice in the *in vivo* chemotherapy studies. An observation was made that the usual diluent used for our virus inoculum, Pucks balanced salt solution (PBSS) was more yellow than usual—an indication of an acidic pH. On some occasions, it has also been noted that when an unusually large number of mice are infected at the same time, the ice bath in which the virus inoculum is placed sometimes became quite thawed. Studies were run *in vitro* to determine if pH or possibly the medium itself, or the temperature in which the diluted virus was held, might affect the virus titer.

Materials and Methods

Virus: Adames strain PTV as described earlier was used.

Cells: Rhesus monkey kidney (LLC-MK_2) cells were used, using standard growth medium(Earles minimum essential medium [MEM] + 5% fetal bovine serum [FBS], $NaHCO_3$ and no antibiotic).

Animals: Three week-old C57BL/6 mice (Simonsen) were used.

Experiment Design: In the experiments, our standard stock PTV was diluted equally in PBSS at pH 6.5, PBSS at pH 7.5, and MEM with 2% FBS at pH 7.5. The three virus/diluent mixtures were then divided in half, with one-half remaining at room temperature, the other in an ice bath. Each diluted virus was then titrated by inoculating varying 10-fold dilutions onto monolayers of LLC-MK_2 cells and incubating a standard period of time to allow CPE to occur. The experiment was performed in 96-well tissue culture plates.

Results and Discussion

The results are summarized in Figure XXI-1. In A, the effect of each diluent held in the ice bath is seen. The virus diluted in MEM with FBS had the greatest titer, approximately $10^{7.5}$ cell culture 50% infectious doses (CCID50) per 0.1 ml. The titer remained approximately the same throughout the incubation period. The virus in PBSS pH 7.5 was approximately one log lower in titer, and the titer declined a full additional $\log_{10}$ upon one hour incubation in the ice bath. The PTV in the PBSS pH 6.5 had nearly 4 logs less virus than that in the MEM, and this virus declined one-half $\log_{10}$ during the hour incubation.

As seen in B, these same relative observations were seen when the virus was held at room temperature. Comparing all the data in C, it appears that PTV placed in any diluent kept at room temperature was lower in titer in all diluents than that kept cold.

These data clarify much of our problems of inadequate virus-induced deaths seen in occasional sets of virus control animals in our chemotherapy studies.

A titration was performed in 3 week-old mice using our stock PTV diluted in MEM + FBS instead of PBSS. The results, seen in Table XXI-1, indicate the virus was highly lethal to these mice, with an LD50 of $10^{-4.3}$. No "window" of infectivity was seen in this study.

Conclusions

The PTV used in our chemotherapy experiments was more infectious when diluted in tissue culture medium (MEM) with FBS than in PBSS. The virus was quite sensitive to acid pH and to warm temperature. An animal titration using PTV diluted in MEM + FBS and kept cold indicated the virus to be lethally infective with no "window" of infection as seen previously using PBSS as diluent.

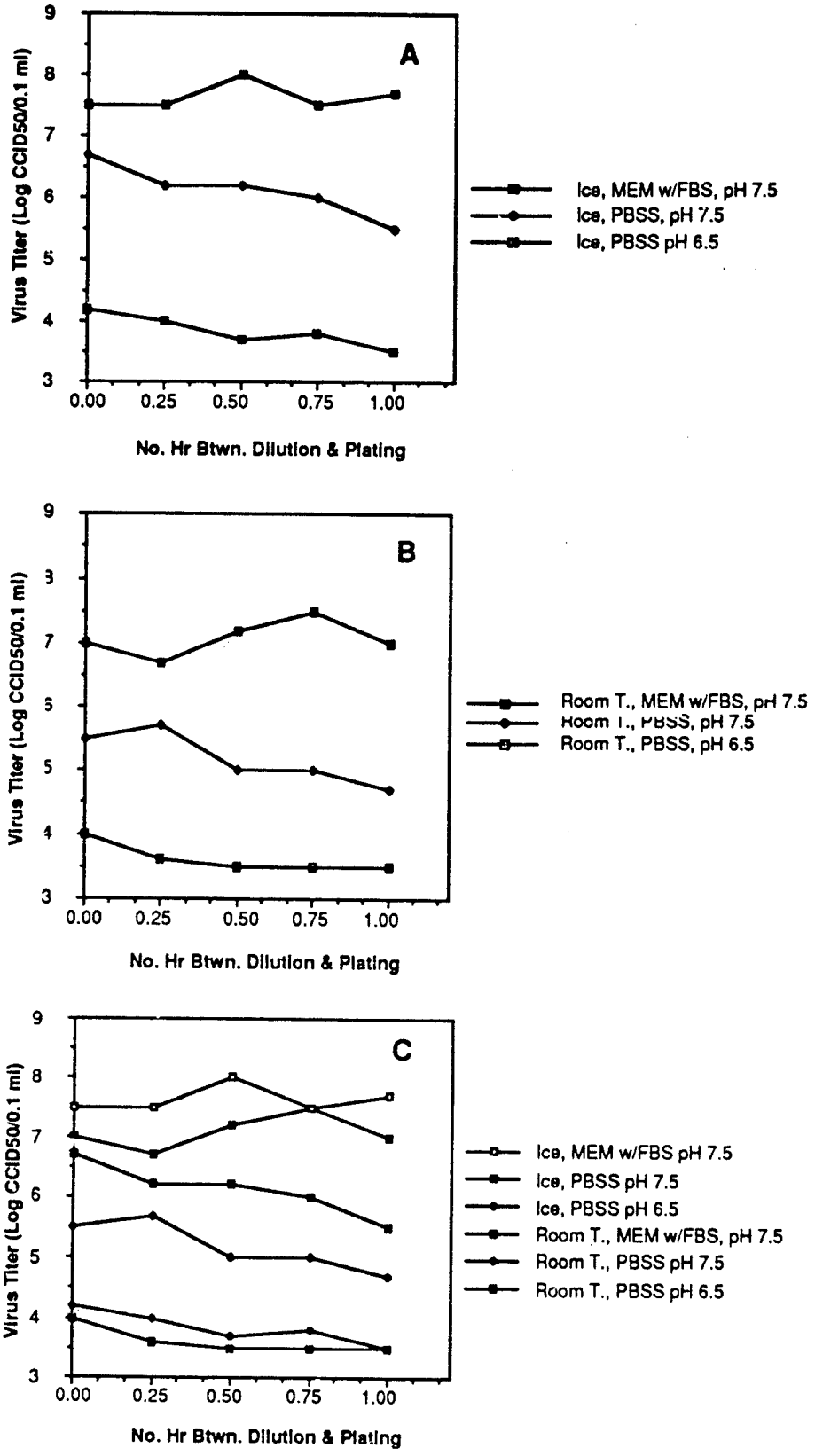
Table XXI-1. Animal Infectivity of PTV[a] Using Minimum Essential Medium Containing 2% Fetal Bovine Serum as Diluent.

Virus Dilution	3 Week-old Mice % Survivors	Mean Surv. Time[b] (days)
$10^{-1.0}$	0	3.8
$10^{-1.5}$	0	4.4
$10^{-2.0}$	0	4.0
$10^{-2.5}$	0	4.6
$10^{-3.0}$	0	4.0
$10^{-3.5}$	0	4.0
$10^{-4.0}$	0	5.8
$10^{-4.5}$	20	4.0
$10^{-5.0}$	100	>21
	LD50 = $10^{-4.3}$	

[a]Pool designated as PTA/4LLC 8-19-87.

[b]Mice held through 21 days.

Figure XXI-1. Influence of Diluent and Temperature on PTV Infectivity



XXII. STUDIES TO DETERMINE THE *IN VITRO* ANTIVIRAL ACTIVITY OF IMMUNOMODULATORS AVS2149 AND AVS1761

Introduction

The two compounds AVS1761 (poly IC•LC) and AVS2149 (ampligen, poly I•poly C12u) have excellent *in vivo* anti-PTV activity. It is presumed that some, if not all, of that activity is due to their interferon (IFN) inducing characteristics. These compounds are not active in the standard antiviral screening procedure. It was decided that they should be observed under conditions designed to induce IFN *in vitro*. Studies were therefore run to determine *in vitro* anti-PTV activity under conditions designed to allow IFN production in the test cells.

Materials and Methods

Virus: Adames strain PTV as used in the standard antiviral screen was used.

Cells: Rhesus monkey kidney (LLC-MK_2 derivative) cells were used.

Experimental Design: Cells were exposed to different concentrations of each compound for 2, 3, or 4 hr prior to exposure of the cells to PTV. In one-half of the experiments, compounds were removed after the above exposure times and the cells were rinsed 1x with MEM without serum immediately prior to placing virus on the cells. In the other half of the experiments the compounds were left on the cells throughout the experiments. When CPE due to the PTV was near 100% in the virus controls all wells were observed microscopically and the extent of the viral CPE was graded and relative activities calculated as in normal *in vitro* antiviral studies. All experiments were run with ribavirin (AVS-01) as a control.

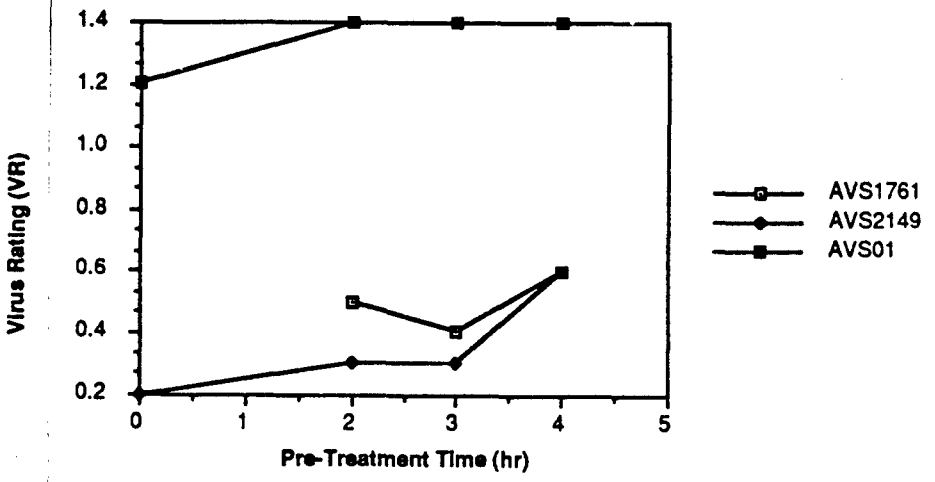
Results and Discussion

When the compounds were left on the cells throughout virus exposure the anti-PTV activity was roughly proportional to the time of virus pre-exposure, as seen by the virus ratings plotted in Figure XXII-1. Data plotted from ED_{50} or TI_{50} calculations provided similar results. Neither compound had discernable activity when compounds were removed prior to virus exposure. These exposure times should have been sufficient to trigger IFN production in the exposed cells. However, the competence of these cells for production of IFN is not known to us. It may be that these cells are poor IFN producers or that they respond poorly to protection by IFN exposure. Studies to determine the IFN-inducing ability of these compounds in L-cells, a mouse-derived cell line known to produce and respond exquisitely to IFN, are planned.

Conclusions

Treatment of LLC-MK_2 derivative cells with either AVS1761 or 2149 up to 4 hours prior to infection with PTV did not protect the cells from the virus if the compounds were removed prior to virus exposure. Treatment of the cells with either compound prior to and continued during virus exposure provided some protection against virus. The protection was proportional to the pre-virus treatment time.

Figure XXII-1. Virus Ratings of AVS-1,-1761, and -2149 when the Compounds were Exposed to LLC-MK_2 Cells for Various Times Prior to PTV.



XXIII. STUDIES TO DETERMINE THE NATURE OF THE INTERFERENCE OF INFECTIVITY OF PUNTA TORO VIRUS

Introduction

Titrations of many of the PTV pools prepared in tissue culture as well as those prepared from animal tissue produced a "window" effect of lethality in mice. The lowest dilutions of virus would not kill, or killed only a small percentage of mice injected with the preparation. The next higher dilutions typically killed 100% of the mice injected and the highest dilutions killed fewer mice in direct proportion to the dilution of the virus. These observations led us to conclude that defective interfering (DI) virus particles may be responsible for the protective effect seen at the highest concentrations of virus used. It was decided that a small plaque isolate of the virus may provide more of the DI particles than the normally used large plaque isolate since DI particles could contribute to the reduction of plaque size seen in cell culture. Studies were run to compare pathogenicity of virus pools prepared in similar ways from large plaque and small plaque isolates of PTV.

Materials and Methods

Virus: Large plaque and small plaque isolates of Adames strain PTV were used at various multiplicities of infection (m.o.i.) to infect cells and virus pools were prepared at various times after virus infection.

Cells: Rhesus monkey kidney(LLC-MK_2 derivative) cells were used, using standard growth medium (Earles minimum essential medium [MEM] + 5% fetal bovine serum [FBS], $NaHCO_3$ and no antibiotic).

Animals: Three week-old C57BL/6 mice (Simonsen) were used.

Experimental Design: Virus pools were diluted in PBSS and 0.2 ml was injected into each mouse. Five mice were used for each 10-fold dilution of virus. The mice were observed for death over a period of 21 days. The virus pools were also titered in cell cultures in 96-well tissue culture plates with 0.1 ml of virus in each well. Four wells were used for each 10-fold dilution of virus. After the effects of virus diluent had been noted in mice, the small plaque pools of virus were re-titered in mice but MEM, 2%FBS was used for diluent in place of PBSS.

Results and Discussion

Some differences were seen in the "window" of lethality when large plaque and small plaque pools were titered in mice and PBSS was used as diluent. Data from three PTV pools initiated by 0.01 m.o.i. and harvested at 48 hr are represented graphically in Figure XXIII-1. However, when the corresponding small plaque PTV pools were diluted in MEM with FBS, the interference previously seen at low dilutions in PBSS was eliminated, as seen in Figure XXIII-2. At low dilutions, there seemed to be sufficient virus inactivated by the PBSS diluent to effectively interfere with the lethality of the virus that remains active. When diluted in MEM with FBS, titers of the different small plaque PTV pools were directly proportional when titered in cell culture or in mice (see Figure XXIII-3).

Conclusions

Interference to PTV lethality seen at high doses of virus when PTV preparations were titered in mice was found to be due to the virus diluent used. There seems to be sufficient virus inactivated by PBSS to cause significant interference of the mortality one would expect at low dilutions of PTV pools. Use of MEM with FBS can eliminate such interference.

Figure XXIII-1. Comparison of the Lethality of Large Plaque vs Small Plaque PTV Preparations Diluted in Pucks Balanced Salt Solution

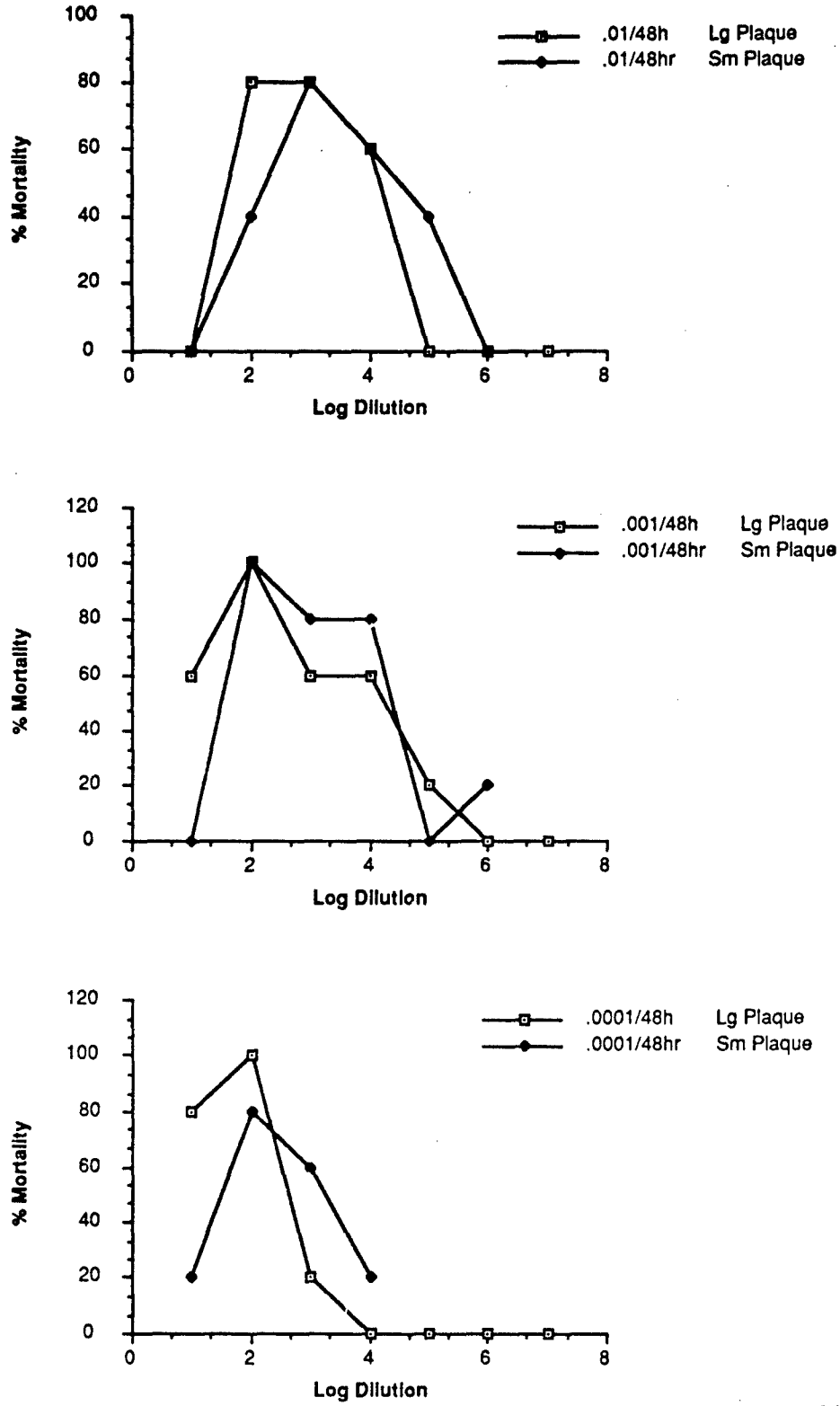


Figure XXIII-2. Comparison of the Lethality of Small Plaque PTV Preparations Diluted in Pucks Balanced Salt Solution vs MEM with FBS.

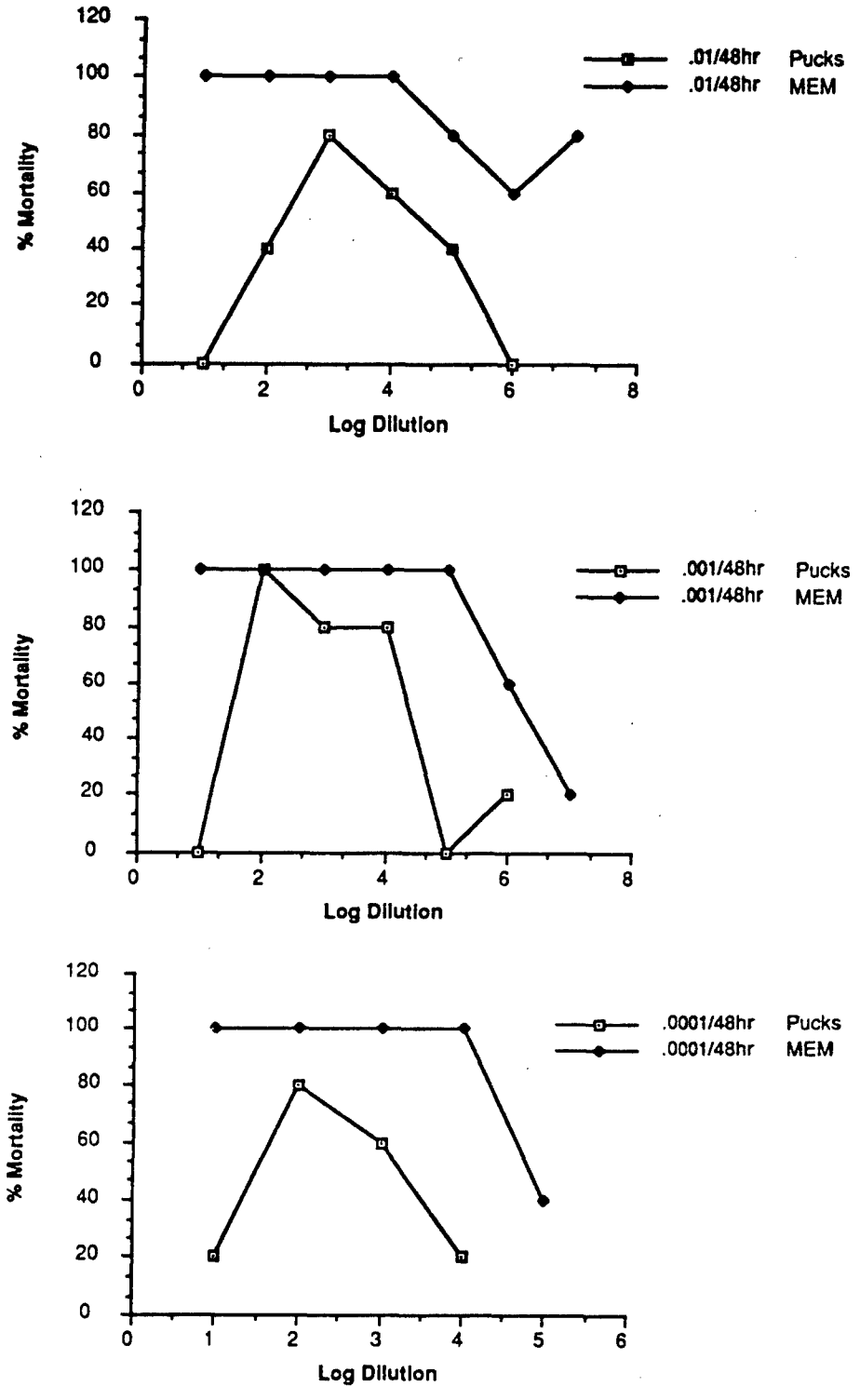
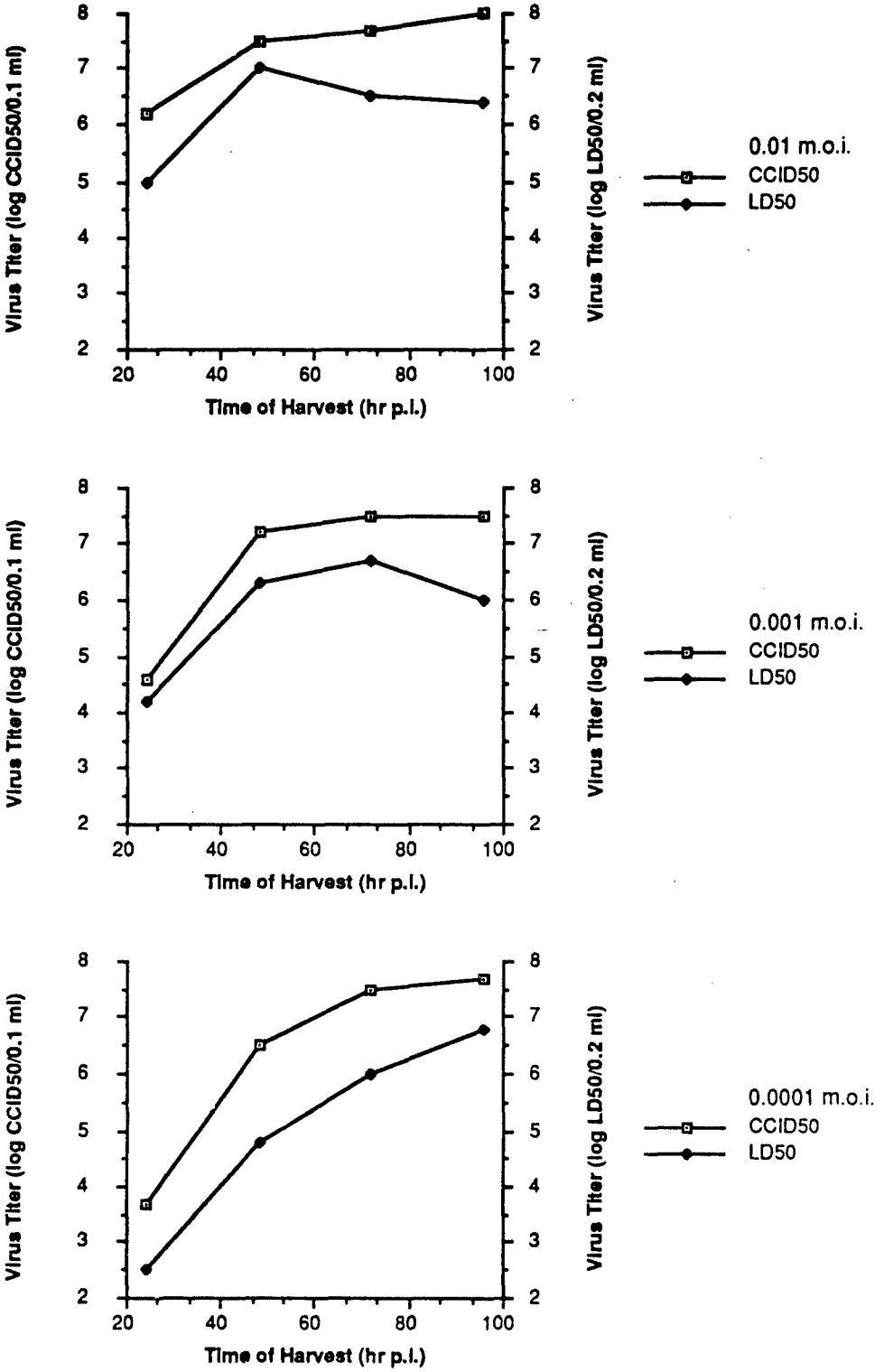


Figure XXIII-3. Comparison of Titers of Small Plaque PTV Preparations when Titered in Cell Culture or in Mice.



XXIV. PRESENTATIONS AND PUBLICATIONS

Presentations

1. Huffman, J.H., R.W. Sidwell, B.B. Barnett, and D.Y. Pifat. Effects of a 3-carboxamidine derivative of ribavirin on phlebovirus infections. Abst. 16, IVth Annual Conference, Inter-American Society for Chemotherapy, Clearwater Beach, Florida. January 10-13, 1988.
2. Sidwell, R.W., J.H. Huffman, B.B. Barnett, M. Kende and D.Y. Pifat. 1988. Effects of a series of immunomodulators on experimental phlebovirus infections. Presented 13 April, 1988 at the Second International Conference on Antiviral Research, Williamsburg, VA. Antiviral Res. 9:125, 1988.
3. Pifat, D.Y., R.W. Sidwell and P.G. Canonico. 1988. Toxicity evaluations of 1-ß-D-ribofuranosyl-1,2,4-triazole-3-carboxamidine HCl (AVS206) in rhesus monkeys: Comparison with ribavirin. Presented 13 April, 1988 at the Second International Conference on Antiviral Research, Williamsburg, VA. Antiviral Res. 9:136, 1988.
4. Gabrielsen, B., M.A. Ussery, P.G. Canonico, G.R. Pettit, E.M. Schubert, and R.W. Sidwell. 1988. Anti-RNA-viral activities of phenanthridones related to narciclasine. Presented 11 April, 1988 at the Second International Conference on Antiviral Research, Williamsburg, VA. Antiviral Res. 9:97, 1988.
5. Huffman, J.H., R.W. Sidwell, R.K. Robins, G.R. Revankar, and D.Y. Pifat. In vitro and in vivo Phlebovirus inhibition by nucleosides related to ribavirin. To be presented at the Round Table on Nucleosides, Nucleotides, and Their Biological Applications, Orange Beach, Alabama, October 2-5, 1988.
6. Sidwell, R.W. 1988 Effects of AVS compounds on experimental PTV infections. Seminar presented to USAMRIID, July.
7. Ussery, M.A., D.Y. Pifat, J.T. Rankin, P.G. Canonico, R.W. Sidwell, G. Tignor, and W.M. Shannon. 1988. Broad-spectrum activity of AVS206, a derivative of ribavirin with reduced erythrotoxicity. Presented at the 28th Interscience Conference on Antimicrobial Agents and Chemotherapy, Los Angeles, CA, October. Abst. 104.
8. Sidwell, R.W. 1988. Immunomodulators as antiviral agents. Presented at the 5th Annual Congress of the Inter-American Society for Chemotherapy, Buenos Aires, Argentina, November.
9. Sidwell, R.W. 1988. Ribavirin, a broad spectrum antiviral agents: Review of activity and clinical utility. Presented at the 5th Annual Congress of the Inter-American Society for Chemotherapy, Buenos Aires, Argentina, November.
10. Sidwell, R.W., J.H. Huffman, H. Renis, M. Kende, and J. Huggins. 1989. In vivo antiviral activity of bropirimine, an orally effective immunomodulator. To be presented at the Annual Meeting of the American Society for Microbiology, New Orleans, LA, May.

Publications

1. Sidwell, R.W., J.H. Huffman, B.B. Barnett, and D.Y. Pifat. (1988) In vitro and in vivo phlebovirus inhibition by ribavirin. Antimicrob. Ag. Chemother. 32:331-336.
2. Sidwell, R.W., J.H. Huffman, D.L. Barnard, and D.Y. Pifat. (1989) Effects of ribamidine, a 3-carboxamidine derivative of ribavirin, on experimentally induced *Phlebovirus* infections. Antiviral Res., (in press).
3. Huffman, J.H., R.W. Sidwell, R.K. Robins, G.R. Revankar, and D.Y. Pifat. 1989. In vitro and in vivo Phlebovirus inhibition by nucleosides related to ribavirin. Nucleotides and Nucleosides (in press).